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pH CHANGES IN RABBIT AND HUMAN STRIATED MUSCLE AFTER CONTRACTION[1]

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As early as 1865 the conception that alkaline substances were produced in muscular contraction along with lactic acid was current. Ranke (9) suggested that creatine was an alkaline fatigue product. Attempts to measure directly the reaction changes due to activity have been limited to two types of experiment: first, with apparatus measuring pH changes through the carbon dioxide intake or output of isolated muscle; and second, through the application of intravital indicators.

Using the first method Lipmann and Meyerhof (5) found that the primary change in contracting frog's muscle was alkaline, and that no acidity could be demonstrated by their method until several volleys of stimuli had been given. Margaria and collaborators (7) by the injection of indicators in the intact frog before isolating the muscle, showed that contraction was accompanied by an alkaline change which they estimated to be approximately 0.5 pH.

Our perusal of the literature has revealed no observations of reaction changes in muscle with circulation intact. Since it is known that the contraction products diffuse out of the muscle, an intact circulation would tend to minimize reaction changes. With the recent advances in the use of the glass electrode in determining pH electrometrically, a new approach to the problem is possible.

METHOD. Since we desired to determine changes within or as close to the muscle cell as possible, a capillary glass electrode was used. The capillary could be inserted intercellularly and would therefore at least detect changes in the tissue fluids bathing the muscle cells. Electrodes with a sensitive tip in the form of a capillary were described and used for

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determination of pH of resting tissues by Voegtlin et al. (11). We modified their technique by including the grid calomel half cell in the electrode as had been done before with electrodes of other shape (13).

Corning glass 015, which is standard for glass electrodes, is used in making the capillary. The shank of the electrode is made of ordinary soft glass. Kroenig's glass cement is used to cover the region of transition between thick glass and capillary. The entire electrode, except for the capillary, is coated with ceresin to prevent contact potentials due to wetting, etc., during use. Such electrodes after several days of soaking have a small asymmetrical potential, and give from 40 to 50 mv/pH. Calibration of the electrodes on standard buffer solutions at the temperature of the animal is done before and after each experiment.

For measuring pH potential we required an electrometer of marked stability. A vacuum tube voltmeter employing a D 96475 Western Electric tube in a balanced electrometer circuit was constructed. The circuit is diagrammed in figure 1. A Leeds and Northrup type 2500b galvanometer whose sensitivity is 5×10^{-10} amp/mm. was used. The resulting electrometer is very stable at a sensitivity of 40,000 mm/volt. It can be used by the null point method, or over short ranges as a scale potentiometer. Perfect linearity has been repeatedly demonstrated over a range of 0.25 pH with our electrodes. At 40 mv/pH one millimeter on our scale equals 0.0005 pH.

A rabbit was anesthetized with sodium amytal intravenously. The fur was cut from the two hind legs and tail. The gastrocnemius muscle and sciatic nerve were exposed on one side, care being taken to prevent interference with circulation. The nerve was ligated doubly as high in the thigh as possible and then cut between the ligatures. The entire fur covered surface of the animal was wrapped in moist cloths and the animal was placed between rubber sheets on a small operating board. By this procedure drying was prevented and static effects due to hair movement were eliminated. The peripheral end of the cut sciatic nerve was placed in a shielded stimulating electrode. One toe of the opposite hind foot was incised deeply enough to obtain a free flow of blood.

Board and animal were then placed in an electrically shielded cage which was kept at a constant temperature of 96°F. Within this cage the separately shielded tube, the ground calomel half cell, and the capillary electrodes were kept at all times. The foot with cut toe was inserted into a separatory funnel filled with mammalian Ringer's solution which through a stop-cock made liquid junction with the saturated KCl of the ground calomel half cell. The capillary of the electrode was then inserted into the belly of the muscle parallel with its fibers. Stimulation by drying was prevented by covering with a pad of gauze moistened with isotonic solution. Contact was made from the grid lead of the tube to the platinum lead of the electrode. Figure 1 shows the resulting circuit.

It was then possible, using the null point method, to balance the potentiometer output of the glass electrode system with the type K potentiometer. During the first 30 to 40 minutes there was a rapid fall, and a prolonged slow rise of the millivoltage reading to a relatively constant level. This phenomenon has been reported before (12). When the readings had become constant the instrument was used as a scale potentiometer unless the changes exceeded 0.20 pH.

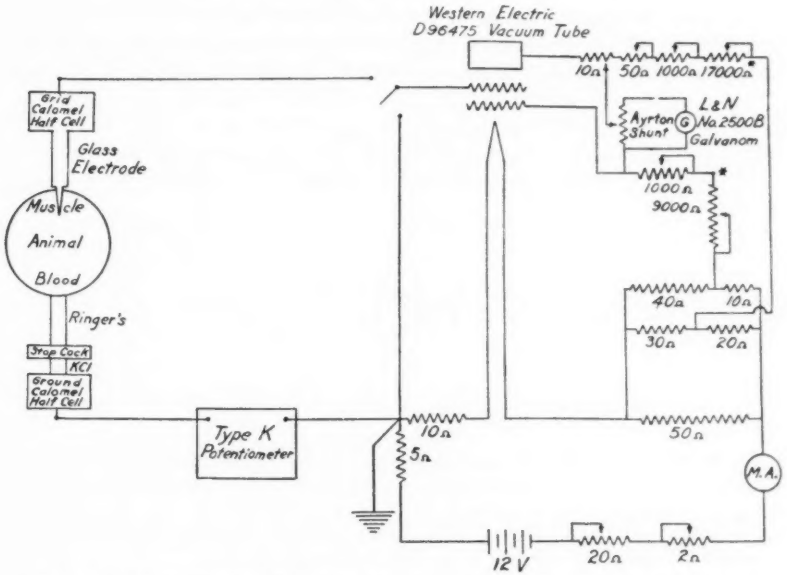

Fig. 1 is a diagram of the balanced electrometer circuit of Barth, as modified by E. J. Bernet, Dept. of Physics, University of Wisconsin. A separate Leeds & Northrup No. 2420 galvanometer in conjunction with a Weston standard cell is used to balance the Type K potentiometer which is powered by an Eveready Air Cell.

* These resistances are banks of 1,000 Ω resistors which may be tapped at each 1,000 Ω.

Before the muscle can be stimulated the electrometer must be shunted. pH changes cannot be observed during the stimulation because the glass electrode will transmit any potential change, whatever its source, and while we have carefully eliminated stimulus escape, it is impossible to eliminate the cumulative effect of the action spikes. While the size of the individual spike potential may be quite small on our scale the long period of the moving coil galvanometer will not permit it to recover before the next spike drives it farther from its resting point. During the time required for a number of stimuli such a cumulative effect would be quite large. Since this accumulation effect on the galvanometer is impossible

if the electrometer is shunted readings may be resumed as soon as stimulation ceases. Thus during a period approximately 6 to 10 seconds longer than the period of the stimulus no readings are taken. Controls were of course necessary to show that after potentials were not included in our results. Stimuli are delivered at a constant rate of 5 per second from an 0.1 microfarad condenser at 1½ volts for whatever period is desired.

By this method the pH of the tissue fluid between the muscle cells can be measured before and after isotonic contractions of striated muscle stimulated through its nerve in the anesthetized animal.

RESULTS. In more than 30 rabbits we have seen a typical pattern response of muscle pH after contraction. This pattern consists of an alkaline

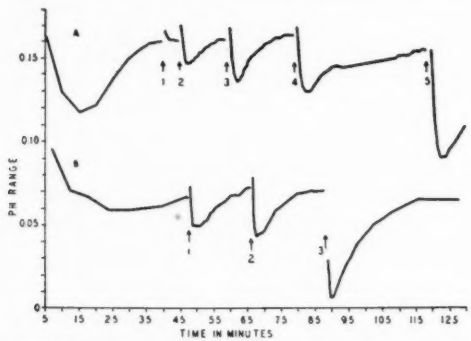

Fig. 2. Plot of pH changes in gastrocnemius muscle of intact rabbit under amytal anesthesia. Muscle is stimulated through cut nerve at a fixed rate of 5 per second. pH range is from an arbitrary zero to indicate size of variations. No observations can be made during stimulation. Time is measured from insertion of glass electrode in muscle. Typical experiments illustrating pattern of response.

A. 1.—5 sec. stim. 2.—10 sec. stim. 3.—15 sec. stim. 4.—30 sec. stim. 5.—60 sec. stim.

B. 1.—10 sec. stim. 2.—15 sec. stim. 3.—30 sec. stim.

change which is receding as our readings are resumed, followed by an acid change which rapidly reaches a peak, then requires a considerable period for neutralization. Both the alkaline and acid phases are graded with length of stimulus. A very short stimulus of 5 seconds (25 stimuli) may be unaccompanied by any acid change, the alkaline change being gradually neutralized and the plateau resumed. A prolonged stimulus, such as 30 or 60 seconds (150 or 300 stimuli), sees the alkaline change already eliminated by the time our readings are resumed.

Figures 2 and 3 show stimulation experiments in graphic form. The pH scale is from an arbitrary zero since our interest is in change, not in absolute value. Suffice it to say that the absolute value of the plateau line varies from 7.40 to 7.55, in good agreement with the figures published

by Voegtlin et al. (12) for resting rat muscle pH obtained with the capillary glass electrode.

Figure 2A and B shows typical experiments illustrating the variability of response in different animals. Figure 3A is the graph of an experiment in which the muscle pH changes per second of stimulation were unusually marked, ten seconds' stimulus producing a marked alkaline and acid change, 20 seconds' stimulus completely out-running the alkaline phase before our observations were resumed. In figure 3B the extent of pH change per second of stimulation is markedly depressed, probably due to the passage of more than five hours since cutting of the sciatic nerve. It is to be noted that a 120 second stimulus (600 stimuli) was necessary before the alkaline phase was eliminated by the time observations were resumed.

The effect of deep anesthesia, depression of respiration by pneumothorax, and death, on the muscle pH is shown in figure 4. The immediate post mortem fall of pH is usually within a range of 0.20 to 0.30 pH.

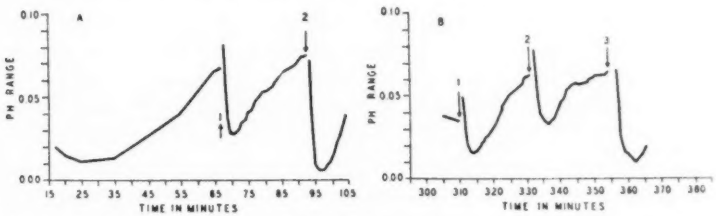

Fig. 3. Conditions same as in figure 2.

A. Graphic pH record of an experiment in which the stimulus was especially effective. 1.—10 sec. stim. 2.—20 sec. stim.

B. Graphic pH record of an experiment in which muscle responses were depressed, probably due to the passage of more than 5 hours since cutting of the sciatic nerve. 1.—30 sec. stim. 2.—60 sec. stim. 3.—120 sec. stim.

In a number of animals lactic acid formation was prevented by administration of purified sodium iodoacetate (100–150 mgm/kgm.) intravenously. It has been reported before that this drug causes rigor of the musculature in maximum alkaline state. Contraction of the muscles during the few minutes before rigor sets in is accompanied by only an alkaline change as is shown by figure 5.

Since the magnitude of the pH change with any given stimulation varies due to numerous causes, quantitative results must be based on averages of trials in many animals. Table 1 gives such averages. The alkaline phase becomes greater and lasts longer with an increasing stimulation period up to 20 seconds (100 stimuli). More than 20 seconds' stimulus on the average results in smaller and more transitory alkaline phases until between 30 and 60 seconds' stimulation the alkaline phase has disappeared by the time our observations are resumed. The acid change on the other

hand is not evident with 5 seconds stimulation but increases with increasing stimulus. By our method stimulus to fatigue results in an acid change whose magnitude varies from 0.3 to 0.4 pH. Not enough data from stimulation periods above 30 seconds have been obtained to include in this summary, although results from such periods are shown in the figures. It

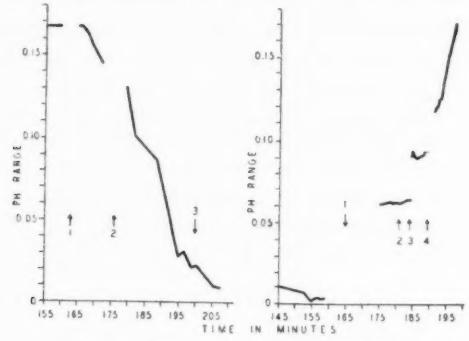

Fig. 4 Fig. 5

Fig. 4. Illustrates the effect of anesthetic depression, and death by asphyxia on pH of gastrocnemius muscle of rabbit. 1.—Amytal 120 mgm. intraperitoneally. 2.—Pneumothorax. 3.—Gasping ceased.

Fig. 5. Illustrates blockage of acid changes in the gastrocnemius of a rabbit due to sodium iodoacetate given by vein. Stimulation at standard rate of 5 per second. 1.—Na iodo. 100 mgm. 2.—Respiration ceased. 3.—10 sec. stim. 4.—70 sec. stim. to fatigue.

TABLE 1

Average pH changes in gastrocnemius muscle of intact rabbit due to isotonic twitches at a rate of 5 per second

NUMBER OF ANIMALS	NUMBER OF TRIALS	PERIOD OF STIMULATION	pH CHANGE		TIME OF ALKALINE PHASE	RECOVERY TIME INCLUDING STIMULUS
			Alkaline	Acid		
		seconds			*seconds*	*minutes*
6	10	5	0.013		210	
17	35	10	0.014	0.019	63	10
12	15	20	0.027	0.035	89	13
11	15	30	0.007	0.063	60	19
		To fatigue		0.350		

is to be noted that degree of acidity increases with longer stimulation throughout.

In the course of our experiments we have occasionally used frogs, rats, and guinea pigs. The general pattern of response was the same in all these experiments.

DISCUSSION. We have found no reference to the effect of extraneous potential on the glass electrode system, and in the early phases of this work in 1935 we began to suspect, from the peculiar responses during contraction, that action currents might be affecting our results. To test this possibility we placed two leads from a single dry cell (with variable resistance) in our separatory funnel with Ringer's solution in which a glass electrode was suspended. Whatever potential was run from the dry cell through the Ringer's solution summated directly with the pH potential reading. On breaking the dry cell circuit the potential rose or fell, within the period of our galvanometer, to the pH potential previously present.

A similar extraneous potential effect was produced by rubbing a glass rod lightly through the fur of a dry rabbit while the electrode was in the muscle. Out of these observations came the technique of wrapping the fur covered surface in wet cloths, to prevent static effects due to movement such as respiration or movement of the foot in response to sciatic stimulation.

The period of changing potential which lasts 30 to 40 minutes after the capillary is inserted in the muscle was observed by Voegtlin and his coworkers (12) in their experiments on resting muscle pH in rats. They reported that this phenomenon did not occur in other tissues but was characteristic of muscle tissue. They attribute it to actual pH changes due to injury of muscle cells. It was our conception that while part of this might be actual pH change, a large part must be pure injury potential. With this in mind we inserted a Zn-$ZnCl_2$-Ringer's isoelectric electrode of capillary shape into the muscle and recorded the potential resulting. Since this potential was opposite in sign to the pH potential it would reduce the voltage output of the glass electrode. The injury potential gradually disappeared over the same period of time as was required for the pH potential to reach a resting base-line. Its magnitude was such as to account for the difference between potential on insertion of the electrode and final base-line.

The ability of the glass electrode system to be affected by extraneous potential necessitated control experiments to show that the changes after contraction were in reality pH changes, and not changes due to some other phenomenon. We have mentioned above that isoelectric electrodes were inserted in the muscle in the same manner as were the glass electrodes. In this case there was no potential change after stimulation—the reading remained the same. In a series circuit such as is used here the smaller the resistance of the electrode the better chance it has to bring small potential effects to the electrometer. Since the resistance of the glass electrode is several thousand times that of the isoelectric electrode any potentials which might be adding to the pH potential after contraction would certainly show up when the isoelectric electrode was substituted for

the glass electrode. Since no potential changes were recorded by our system when the isoelectric electrode was used we conclude that no extraneous potentials are affecting our results.

Again, a glass electrode was placed in the subcutaneous tissue of the leg where a movement around the electrode would occur when the muscle was stimulated. Contraction of the muscle resulted in no change of reading. Since extraneous potentials would spread from the muscle to the subcutaneous tissue this phenomenon is another demonstration that after potentials are not recorded by our system. In another type of control experiment passive manipulation of the leg to give very forceful movement of the muscle around the electrode failed to evoke any change.

That the magnitude of the response to any given stimulus varies in different animals, or in the same animal at different times, may be due to a number of factors. First, the state of anesthesia may influence the response. Second, the effectiveness of the nerve stimulus may vary with time after the nerve has been cut. (See fig. 3B.) Third, it is a common observation in analysing muscles for lactic acid after contraction that the changes show wide variability in different experiments.

The ability of the muscular system to resist pH change with moderate amounts of work is well shown by our results, since 150 twitches produced an average change of only 0.06 pH. It should be remembered, however, that these muscles are unweighted, also that the changes here recorded are probably those occurring in the tissue spaces, not necessarily identical with those in the muscle fiber itself. pH changes in the blood of man after fatiguing exertion have been reported by Laug (3). He found changes of 0.3 pH which are similar to our changes of 0.35 pH in fatigued muscle.

The time required for the return of the muscle pH to its resting level after contraction compares well with the findings of other investigators, who have determined the rate of removal of contraction products from blood and muscle. With various rates and durations of contraction many authors have reported that recovery is a matter of a few minutes to hours, depending on the product considered, and the amount and rate of work done.

All theories of muscle chemistry agree that the principal alkaline product of contraction is creatine, the principal acid one lactic acid. According to the Meyerhof cycle creatine alkalinity should predominate in initial activity, and the concentration of lactic acid should rise, though not proportionately, with increasing amounts of rapid work. This would produce pH changes of the pattern here reported.

The fact that maximum acidity is not reached until several minutes after cessation of contraction may be caused by the delayed lactic acid formation first reported by Embden (2). However, the occurrence of

maximum acidity in the fiber may be earlier than the appearance of maximum acidity in the tissue space as time may be required for diffusion to occur. Either or a combination of these phenomena may be responsible.

Under iodoacetic acid creatine is formed as before, and also ammonia is released from the breakdown of adenosine triphosphate (Parnas, 8). These processes in the absence of lactic acid produce the alkaline changes after contraction, and the maximum alkaline state reported by Voegtlin et al. (12).

One further series of controls seemed to be indicated to assure us that the pattern of reaction seen was present in the normal animal. We desired to eliminate anesthesia, artificial stimulation, and widespread exposure of the muscle.

pH CHANGES AFTER VOLUNTARY CONTRACTION IN HUMAN STRIATED MUSCLE. METHOD. Adult male subjects were seated in a chair with the left arm pronated upon the arm-rest of an extensor ergograph. The legs of the chair and subject were insulated from the floor by glass and paraffin. Under procaine desensitization of the skin an incision about 4 cm. in length was made through the skin and superficial fascia over the belly of the extensor digitorum communis, parallel with its fibers. Care was taken to render the field completely dry by tying off all small bleeders. The point under the deep fascia where greatest activity occurred when the digits were being extended was found by direct vision. In this locus an incision about 0.5 cm. in length was made through the deep fascia.

A capillary glass electrode was sterilized for 24 hours before use in hexyl resorcinol solution 1:1000. After rinsing with sterile saline its capillary tip was inserted into the muscle parallel with its fibers. The ground connection to the subject was obtained through a saline-blood contact at the left shoulder. A few small scratches sufficiently deep to draw blood were made on the flattest portion of the superior surface of the shoulder. A 2 cm. diameter glass funnel whose exit tube had been cut off was attached firmly to the skin around the scratches with collodion. When this was dry the funnel was filled with saline and a simple glass saline bridge (no bubbles tolerated) made connection from the subject to the separatory funnel filled with saline which was used for ground connection as in the previous rabbit experiments.

Two principal differences between the technique in the rabbit and in the human experiments are that in the latter the electrodes are used at room temperature, and calibration of the electrodes is possible only after use, not before and after. The calibration was made with buffer solutions at body temperature. The electrical set-up and its handling were unchanged.

Contractions were done voluntarily with and without load, and with the circulation either normal or blocked by a pressure cuff on the arm at

greater than systolic pressure. The changes in pH of the muscle, the number of contractions, the amount of work done, and the fatigue sensations experienced were noted.

RESULTS. Four human experiments have been done. In no case did the insertion effect seen in the rabbits occur. There was, however, a constant rise of base-line whose magnitude per minute was almost equal

TABLE 2

Results of four human experiments on reaction changes in the extensor digitorum communis following voluntary activity

The results are grouped in the order of work performed

CONTRACTIONS		LOAD LIFTED	WORK DONE	pH CHANGES		TIME FOR pH RECOVERY	FATIGUE SENSATION	SUBJECT
Rate per second	Duration			Alakaline	Acid			
Normal muscle group								
	seconds	*grams*	*grams/cm.*			*minutes*		
1½	20	0	0	0.023	0		None	G. M.
3	35	0	0	0.018	0		None	G. M.
1	10	200	12,400	0.018	0.007	4	None	W. G.
2	10	200	16,000	0.036	0	3½	None	W. M.
2	15	200	16,200	0.041	0		None	W. M.
2	15	200	21,000	0	0.018	6½	None	W. G.
1	30	200	23,000	0.048	0	5	None	C. M.
2	90	200	89,000	0	0.049	13	Marked	W. M.
2	45	1,200	250,000	0	0.185	14½	Marked	W. M.
2	120	200	310,000	0	0.241	17	Slight	W. G.
Fatigued muscle group								
2	7	200	18,000	0	0.071		None	W. G.
2	15	200	26,400	0.030	0.096	10½	None	W. M.
2	55	200	80,000	0	0.160	13½	Slight	W. M.
Ischemic muscle group								
1	30	200	23,800	0.023	0.089	8½	Pain	C. M.
2	55	200	80,000	0	0.242	14	Pain	W. M.
2	45	1,200	390,000	0	0.708	18	Pain	W. G.

in all cases. Its value approximated 0.0015 pH per minute, and showed no change with the passage of time in the experiments. Contraction changes were superimposed on this rising base-line.

In table 2 are summarised the results from these experiments grouped according to the condition of the muscle: normal, previously fatigued, and with blood supply obstructed. In each of these groups the reaction changes are recorded in the order of amount of work done. Three types

of postactivity reaction change are seen. With brief activity and little work only a rise of pH and return to the base-line resulted. With moderate work a short-lived rise followed by a fall of pH and return to the base-line resulted. With severe work the alkaline phase had disappeared before work ceased, and our observations after work was over revealed only a fall of pH followed by slow return. In table 3 the data from one complete experiment are given as obtained.

The general pattern of response to contraction was identical with that seen in the rabbit. The degree of reaction change was related to the amount of work done as well as to the rate of activity, and the previous condition of the muscle. In a muscle which had already undergone marked fatigue the acidification per unit of work done was considerably

TABLE 3

Complete data from one typical human experiment (Subject W. G.) in order of tasks performed

CONTRACTIONS		LOAD LIFTED	WORK DONE	pH CHANGES		TIME FOR pH RECOVERY	FATIGUE SENSATION
Rate per second	Duration			Alkaline	Acid		
	seconds	*grams*	*grams/cm.*			*minutes*	
2	15	200	21,000	0	0.018	6½	None
1	10	200	12,400	0.018	0.007	4	None
2	120	200	310,000	0	0.241	17	Slight
30 minute rest period							
Tourniquet applied, 150 mm. Hg pressure and contractions started 2 min. later.							
2	45	1,200	390,000	0	0.708	18	Pain
pH recovery did not begin until 90 sec. after work ceased when tourniquet released.							
30 minute rest period							
2	45	1,200	250,000	0	0.185	14½	Marked
2	7	200	18,800	0	0.071		None

increased. (See Fatigued Muscle Group, table 2.) Interruption of the circulation by a tourniquet did not alter the pattern of response, but heightened markedly the degree of acidification, reduced the work ability of the muscle, and brought on typical ischemic work pain which persisted until the tourniquet was released when it disappeared in a very few seconds. The acidification in the ischemic state after work progressed very rapidly for as long as two minutes until the tourniquet was released, whereupon an immediate and rapid neutralization started.

As in the rabbit, with normal circulation maximum acidity was not developed until 1 to 2 minutes after work ceased. While a decreased pH was always present when fatigue sensation occurred, decreased pH was frequently present without the sensation of fatigue. In one individual only slight fatigue was experienced with a fall of 0.241 pH, marked fatigue

in another with only 0.049 pH fall. The latter subject later in his experiment had but slight fatigue with 0.160 pH fall. No evidence of a critical level of pH where fatigue sensation occurred was found, but the number of experiments is too small to be conclusive.

Discussion. The rising base-line present in these experiments renders evaluation of the actual pH of the tissue difficult. In no experiment was the base-line ever below 7.05 nor above 7.45 pH. Two possible hypothetical mechanisms occur to us for this change of base-line: first, an extraneous electrical change; second, alkalinization due to slow carbon dioxide diffusion from the muscle. It does not seem likely that this phenomenon is an insertion effect because of the fact that the rate of base-line change remained the same throughout experiments lasting more than 130 minutes (timed from insertion of electrode). One would expect that the effect of carbon dioxide diffusion would reach an equilibrium before 130 minutes. While this phenomenon may impair the absolute quantitive accuracy of the reaction changes we do not feel that it affects their relative accuracy since its rate is so slow and constant.

Table 2 reveals that the size of the reaction change parallels fairly closely the amount of work performed even in different subjects. It is noted that the reaction changes occurring in these human muscles are much larger than those we reported for the rabbits. However, the conditions are different in that the human contractions were with a considerable load, moved by a rather weak muscle (extensor digitorum communis) whereas in the rabbit the strong gastrocnemius was used unweighted.

As to the sensation of neuromuscular fatigue our results convey the impression that there is little or no correlation between pH level and the sensation, there being not only individual variation but also in the same subject at different times more work with greater fall of pH may evoke less discomfort than a smaller amount of work with less fall of pH. The number of experiments is too small to make these observations more than suggestive.

The influence of applying the tourniquet alone without exercise was not tested over more than the two to two and one-half minutes allowed for arrest of circulation before work started. In two cases a very slow gradual fall of pH occurred, and in one no change was seen. More prolonged periods of resting ischemia before work started were not used in this series of experiments to avoid the onset of fibrillary twitchings which occur on the return of circulation after 15 or more minutes of ischemia as reported by Reid (10). In no case did the period of ischemia used here last more than 6 minutes.

MacWilliam and Webster (6) first differentiated ischemic work pain from the sensation of neuromuscular fatigue. Lewis, Pickering and Rothschild (4) showed that the cause of the ischemic pain was a chemical

entity or a physicochemical process to which they gave the name Factor P. Elliot and Evans (1) in a recent paper have brought forward evidence suggesting that Factor P may be lactic acid or pH change. Our experiments only show that very marked acid changes occur in contractions of ischemic muscle. They suggest that the application of the capillary glass electrode to human experiments on ischemic phenomena should prove fruitful.

SUMMARY

1. A capillary glass electrode has been applied to the study of reaction changes in mammalian striated muscle after contraction in situ in amytalized rabbits and in unanesthetized human subjects.

2. In rabbit and human muscle contraction was followed by an identical pattern of pH change. With small amounts of work only alkaline changes resulted followed by gradual return to resting pH level. Greater amounts of work produced a small and short-lived rise of pH followed by a rapid fall of pH. This acid change was then very gradually neutralized.

3. Increased work results in greater acidification at the rate of activity used in these experiments. The time required for recovery of resting reaction is related to the degree of acidity developed.

4. Sodium iodoacetate heightened the alkaline phase and eliminated the acid phase after contraction in the rabbit.

5. In three human subjects greater acidification per unit of work done occurred in previously fatigued than in normal muscles.

6. Work done by human muscle rendered ischemic by a tourniquet was followed by marked acid changes (a fall of 0.71 pH in one case).

7. The reaction changes recorded are probably those occurring in the tissue fluid between the muscle fibers and are not necessarily identical with changes within the fiber itself.

We are indebted to Prof. J. A. E. Eyster and Dr. M. R. Krasno for helpful assistance throughout the course of these experiments, to Dr. A. R. Curreri for surgical assistance, and to Dr. R. H. Fitch for early suggestions about technique.

Since this manuscript was submitted a paper has appeared by M. Dubuisson (Pflüger's Arch. **239**: 314, 1937) describing the application of the glass electrode to the study of the pH changes in the isolated muscle of the frog after isometric contractions in the anaerobic state.

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THE RESPONSE OF THE PLASMA POTASSIUM LEVEL IN MAN TO THE ADMINISTRATION OF EPINEPHRINE

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In 1932 Bachromejew reported that the injection of epinephrine produces a prompt and marked rise in the level of the potassium in the blood serum of cats. This observation was confirmed by D'Silva (1934, 1936) with cats and rats, by Schwarz (1935) with rabbits, and by Marenzi and Gerschman (1936) with dogs. All these investigators agreed that the response occurred within a minute or two after intravenous injection, that the elevation of the potassium concentration in the serum reached a maximum of 20 to 50 per cent above the normal resting level, and that the effect was transient, passing off more or less parallel to the return of the blood pressure to normal.

It might seem that these studies firmly established the generality of an important relation between epinephrine and the serum potassium. On the basis of this and other evidence, Camp (Camp and Higgins, 1936; Camp, 1937) has proposed the theory that the characteristic physiological action of epinephrine is a result, finally, of the liberation of potassium. However, none of these investigations had been carried out on human beings; in a preliminary report (Keys, 1937a) it was shown that man behaves differently. The present study shows that the epinephrine-potassium relation is not only different in man but is actually reversed from what it is in the laboratory animals previously studied.

Methods. Normal healthy young men were used as subjects. All studies were carried out in the fasting state after at least 30 minutes' rest in bed. Samples of blood were drawn under oil from the median basilic vein, without stasis; they were heparinized under oil at 0°C., and plasma was separated immediately by centrifugation under oil in calibrated hematocrit tubes at 0°C. for 90 minutes at 2800 R.P.M. In general, one blood sample was drawn before the injection of epinephrine, one within ½ to 3 minutes after the end of the injection, and other blood samples at increasingly long intervals during the succeeding hour.

Dilutions (1:3000 or 1:5000) of epinephrine ("adrenalin") chloride in isotonic saline solution were freshly prepared in each case from a standard

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1:1000 solution. The epinephrine was injected intravenously over a period of from 1½ to 3 minutes, the amounts being varied in different experiments from 0.005 to 0.30 mgm. of epinephrine chloride.

Blood pressure and pulse records were continuously recorded; in all cases the systolic blood pressure reached a maximum of at least 190 mm. Hg within 3 minutes after injection, and in most cases rose to more than 200.

Cell volumes were obtained from the hematocrit readings. Oxygen capacity was measured by the standard Van Slyke procedure, plasma total nitrogen by the Kjeldahl method. Water of cells and of plasma was determined by weighing, drying for 24 hours at 105°C. and reweighing.

TABLE 1

Summarized results of studies on H. Z., 19 years old, male, weight 67 kgm.

Received 0.15 mgm. of epinephrine chloride (1.45 cc. of 1:3000 solution) intravenously over a period of 2.5 minutes. (Time for blood samples are in minutes after end of injection of epinephrine.)

TIME OF BLOOD SAMPLE	HbO_2 caps.	Cell	CONCENTRATIONS IN PLASMA						CONCENTRATIONS IN RED CELLS			
			H_2O	Total N	Milliequivalents per kgm. H_2O				Milliequivalents per kgm. H_2O			
					K	Na	Total base	Cl	H_2O	K	Total base	Cl
minutes	*vols. per cent*	*vols. per cent*	*gm./L*	*gm./ 100 cc.*					*gm./ kgm.*			
Before injection of epinephrine*	19.52	45.6	944	1.008	4.17	150.4	161.4	112.4	655	128	170	96.3
0.4		48.8	937	1.064	3.83	156.7	162.6		657	128	173	94.5
1.0		49.4	938	1.040	3.84	150.3	157.5	109.6	666	120	160	
10.2		46.4	938	1.035	3.84	155.3	164.7	112.4	663	129	161	94.1
25.2		43.3	940	1.016	3.89	151.2	164.4	113.0	655	122	170	95.5
47.5	19.36	45.1	942	1.006	4.25	152.0	162.5	112.0	652	133	173	93.2

* After 33 minutes' rest.

The following methods were used for the mineral analysis: Na—Butler and Tuthill (1931); Cl—Keys (1937B); K—Hartzler's modification of the method of Shohl and Bennett; total base—Keys (1936). All mineral analyses were done in duplicate, those for potassium frequently in triplicate.

RESULTS. The first trial experiments gave the surprising result that epinephrine produced no rise in the plasma potassium but rather a pronounced fall. This was abundantly confirmed in careful experiments with serial collections of blood samples. The condensed results from a typical experiment are given in table 1.

Identical studies were carried out on 6 normal men; the mean results

are summarized in figures 1 and 2. It is clear that concentrations in the cells, per unit of cell water, are substantially constant and unaltered by

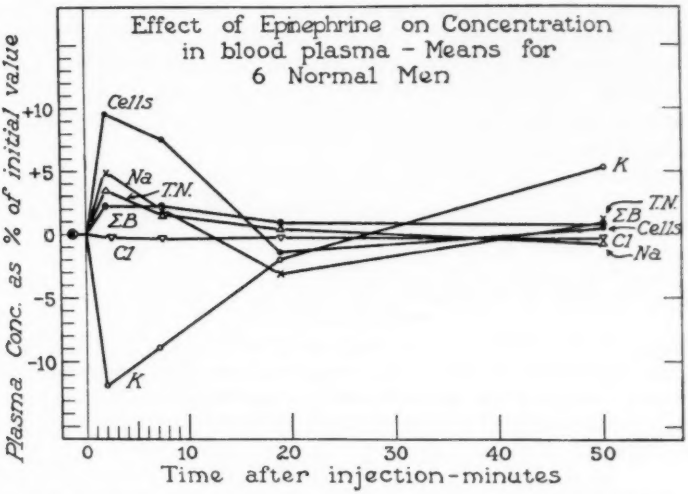

Fig. 1. Effect of the intravenous injection of 0.2 mgm. of epinephrine chloride on the concentrations of red cells, and of sodium, total nitrogen, total base, chloride and potassium in the plasma of normal man. One and a half to three minutes used for injection of the epinephrine. All concentrations calculated per unit of plasma water, expressed as per cent change from the pre-epinephrine level. Mean values from studies on six subjects.

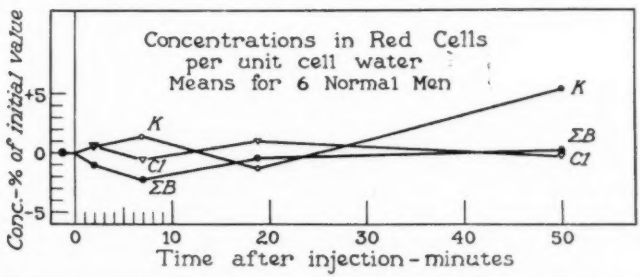

Fig. 2. Concentration of potassium, chloride and total base in the water of the red cells, expressed as per cent change from the pre-epinephrine level, in the same experiments given in figure 1.

the epinephrine except for some rise in the potassium concentration 50 minutes after the injection. In the plasma, on the other hand, there is a consistent and marked drop in the potassium immediately following

the injection of epinephrine. The potassium level returns to normal in about 20 minutes and is significantly above the pre-epinephrine level at 50 minutes.

Aside from the potassium, the blood shows a general hemoconcentration resulting from epinephrine. This is most marked in the rise in the red cell concentration but is also discernible in the level of plasma nitrogen and sodium. The major effects are over in 20 minutes and, at the end of 50 minutes, the concentration of all substances except potassium is back to the pre-epinephrine level.

Similar effects, but more protracted in time, resulted from subcutaneous and intramuscular injections of epinephrine. The same general picture

TABLE 2

Effect of epinephrine on potassium and sodium concentration in blood plasma of unanesthetized rabbits

Samples of blood taken from the left side of the heart before, and 2.5 minutes after, the injection of 0.05 mgm. of epinephrine chloride (0.05 cc. of 1/1000 solution) by ear vein. (Mean values, in milliequivalents per liter, from duplicate analyses.)

RABBIT	WEIGHT	BLOOD SAMPLE	K		Na	
			M. eq./L.	Per cent change	M. eq./L.	Per cent change
	gm.					
A	2,800	Before	5.14		1.63	
		After	6.27	+22.0	1.62	−0.6
B	2,910	Before	4.64		173	
		After	5.92	+27.6	166	−4.5
C	3,150	Before	5.90		170	
		After	6.91	+17.1	172	+1.2

also emerged on injection of as little as 0.005 mgm. of epinephrine chloride and on injection of the largest dose we thought safe to use, namely, 0.3 mgm. into a man of 78 kgm. body weight.

These results for man are diametrically opposed to those reported for cats, dogs, rabbits, and rats. Analytical error was excluded from the present studies by the fact that the triplicate analyses for potassium seldom varied by more than from 1 to 2 per cent. Accordingly, a number of experiments were done on laboratory animals.

Table 2 summarizes the results of three experiments on rabbits. There is a marked rise in the plasma potassium level similar to that reported by Schwarz (1935) in this species. Single experiments on a cat and on a dog likewise confirmed the reports of previous investigators.

COMMENT. There seems to be no alternative to the conclusion that

the effect of the administration of epinephrine on the level of potassium in the blood plasma is to produce, immediately, a marked fall in the case of man as compared with a still more marked rise in cats, dogs, rabbits, and rats. The much larger doses used in most of the animal experiments are not responsible, since a rise was also found in dogs after very minute doses (Marenzi and Gerschman, 1936). Anesthesia was used in most of the animal studies reported in the literature, but again this is not responsible and a rise in plasma potassium after epinephrine is found in unanesthetized animals (Marenzi and Gerschman, 1936; present studies).

It is not proposed to discuss here the details of the mechanism which elevates the plasma potassium in animals; these points are well covered in the papers by D'Silva and by Marenzi and Gerschman. As to the fall of plasma potassium in man, it is as yet only a fact about which little may be said. It is barely possible that the potassium lost from the plasma is taken up by the red cells; the normal disproportion in the total amounts of potassium in the red cells as compared with the plasma makes this very difficult to rule out.

Cloetta, Fischer and van der Loeff (1934) found that central nervous stimulation produces a rise in the potassium concentration in the serum. They presumed this potassium came from the muscle cells, since curare prevented the effect. The mechanism in the case of the rise following the injection of epinephrine into animals may be very different in that only the liver seems to be involved.

D'Silva (1936) and Marenzi and Gerschman (1936) have produced conclusive evidence for the central rôle of the liver in the liberation of potassium on the injection of epinephrine into animals. Lamson (1920, Lamson and others, 1923) previously demonstrated the importance of the liver in the production of increased red cell concentration by epinephrine. What may be the action of the liver in man in this connection remains a question.

Edmunds and Nelson (1925) summarized the earlier literature and attributed the sudden increase in the hemoglobin concentration in the blood following the injection of epinephrine chiefly to a loss of plasma volume (effect of the vasoconstriction). If this were the case, one might expect the plasma protein to increase more or less in proportion to the increase in red cells in terms of units of plasma water. This is not so (figure 1); the red cells rise much more in proportion than does the plasma nitrogen. This may mean either: 1, the circulating blood is enriched with a new and large supply of red cells, or 2, whole plasma, including proteins, is lost from the circulating blood.

In any case it appears that Camp's theory that epinephrine acts by virtue of the liberation of potassium cannot be made into a generalization; in man and in the species of animals reported, the elevation of blood

pressure, vasoconstriction and similar effects are identical but the response of potassium is reversed.

SUMMARY

Previous work on dogs, cats and rabbits is confirmed; the injection of epinephrine produces an immediate marked rise in the level of potassium in the blood plasma. The sodium level is substantially unaltered. This effect of epinephrine is not the result of anesthesia and may be evoked by doses as small as used in man.

In normal man, the intravenous injection of from 0.005 to 0.3 mgm. of epinephrine chloride produces an immediate and marked fall in the level of potassium in the plasma. The potassium concentration returns to near normal in about 20 minutes, and is generally significantly above the pre-epinephrine level after 40 to 60 minutes.

In normal man epinephrine produces an immediate sharp rise in red cell concentration, a smaller but significant rise in the concentrations of sodium and protein in the plasma, and little or no change in total base or chloride.

The concentrations of potassium, chloride and total base in the water of the red cells show no certain response to epinephrine except that the potassium is slightly elevated after 40 to 60 minutes.

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LIBERATION OF ACETYLCHOLINE BY THE SUPERIOR CERVICAL SYMPATHETIC GANGLION AND THE NODOSUM GANGLION OF THE VAGUS[1]

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The formation of a chemical substance at the terminals of the primary neurons is implied in the theories of humoral transmission, although these structures do not have the properties ordinarily associated with secreting organs. In order to surmount the difficulty which is here presented, Lapicque has proposed for muscle that the acetylcholine (A. Ch.) may be derived from the nucleated sole plate, rather than from the endings. An analogous source for the substance in sympathetic ganglion would be the satellite cells, with which the nerve terminals and the dendrites of the ganglion cells seem to be in intimate contact (de Castro, 1926, figs. 3, 6, 13, 16, 19). Experiments have been performed to test this possibility.

As satellite cells are in contact with sensory cells as well as with ganglion cells, obviously the first experiment to be tried in order to test the hypothesis was to look for the production of A. Ch. after stimulation of the vagus ganglion or antidromic stimulation of a sympathetic ganglion. When the experiment was performed, the substance was found; but the conditions of its appearance were such as to suggest it to be a product of metabolism, rather than an agent produced exclusively in a specialized structure for a specific purpose.

The superior cervical sympathetic ganglion and nodosum ganglion of the vagus were perfused in the manner described by Kibjakow (1932), Feldberg and Gaddum (1934), Feldberg and Vartiainen (1934), von Brücke (1935), and Brown and Feldberg (1936 a, b); and the output of A. Ch. was determined with the leech preparation described by Fühner (1918), Minz (1932), and Chang and Gaddum (1933).

Four series of experiments were made, with changes in the technique

[1] The experiments here reported were planned with Dr. H. S. Gasser, who had suggested as a working hypothesis the possibility of the liberation of A.Ch. by satellite cells. Doctor Gasser has taken part in a number of the experiments and offered helpful criticisms of others, for which the author is deeply indebted. Unfortunately, Doctor Gasser has been prevented from following the research in detail, and the present author must take full responsibility for the statements made in this paper.

in each series. All experiments were performed on cats anesthetized with Dial (0.6–0.7 cc. per kgm.).

FIRST SERIES OF EXPERIMENTS. *Technique.* The experiments of the first series followed in every detail the method described by Feldberg and Gaddum (1934) and Feldberg and Vartiainen (1934). A minor change only was made. In order to obtain a larger operating field, the larynx and base of the tongue were extirpated after ligature of the lingual and superior laryngeal arteries on both sides. Supramaximal rhythmic condenser discharges at 4 to 5 per second were employed for stimulation, and their efficacy was checked by observation of the movements of the pupil and nictitating membrane. The bath for the leech preparation was made as small as possible, only 0.7 to 0.8 cc. of fluid being necessary to fill it when the glass holder and the strip (about 15 to 20 segments) of muscle were in position. During the periods between tests, oxygenated plain or eserinized Locke's solution was kept running through the bath.

Results. The first series included seven successful perfusions (I–VII). Three of the experiments (II, IV and VII) yielded results as described by Feldberg and Vartiainen. The specimens of perfusate collected during periods of postganglionic stimulation or of stimulation of the vagus were either inactive, or had the same activity on the leech muscle as control samples collected during periods when there was no stimulation. But the specimens obtained during preganglionic stimulation were always extremely active, two of them producing an effect greater than the A. Ch. solutions at 250 and 200γ per liter respectively. Furthermore it was observed, in agreement with Feldberg and Vartiainen (1934, p. 109), that preganglionic stimulation, delivered after a period of postganglionic tetanus, failed to produce a peripheral response, although large amounts of A. Ch. were released.

In the other four experiments different results were obtained, as considerable amounts of A. Ch. were liberated by postganglionic stimulation. Experiment V (2/9/1937) is typical of this group. The specimens were tested on the leech at various dilutions, 1:1.4 for samples 1, 2, and 4; 1:2.8 for samples 5, 6, and 9; and 1:4.2 for samples 7 and 10. Specimens 3 and 8 were not tested. The sensitivity of the leech strips was assayed with A. Ch. solutions at 5, 10, and 20γ per liter.

Examination of the records shows that at the start of the perfusion (fig. 1, *1 c*, and *3 c*) there was a spontaneous output of A. Ch. which lasted until control sample 4 was collected. The first period of postganglionic stimulation (5) caused liberation of A. Ch. in very nearly the same concentration as was produced by preganglionic stimulation in period 10 (about 80 to 100γ per liter); and after the perfusate had again become negative (sample 6), a second period of postganglionic stimulation (fig. 1, 7 A) likewise brought out a considerable amount of the substance.

Following these procedures it was found that preganglionic stimulation no longer evoked responses of the pupil and nictitating membrane, although A. Ch. was produced in the ganglion (period 10) and the previous postganglionic stimulation had produced maximal contractions.

Inasmuch as the appearance of A. Ch. following postganglionic stimulation was contrary to the observations of Feldberg and Vartiainen, the possibility of direct spread of the stimulating current from the postganglionic to the preganglionic fibers had to be considered. But this explanation was proven to be untenable when the action potentials in the preganglionic fibers were observed oscillographically. Even with the employment of shocks much stronger than were used routinely, no escape occurred. Consequently, the liberation of A. Ch. by impulses conducted antidromically to the ganglion cells had to be regarded as a genuine phenomenon

Fig. 1. Responses of the leech to the perfusate and to standard solutions of A.Ch. (10.5 and 20γ per liter). Experiment V (2/9/1937). The numbers on the record are those of the samples of perfusate. *C*, control; *A*, antidromic stimulation; *D*, preganglionic tetanus. Each horizontal series contains responses of the same strip of leech muscle. *L* and *E* indicate the changes of the bath to Locke's solution and eserinised Locke's solution. Dilutions of the samples indicated in text. In this and the following figures, 1 min. represents one of the small divisions of the time scale.

and it was thought, in agreement with an assumption of Eccles (1936, p. 363), that for some reason the antidromic impulses in the negative experiments had been blocked somewhere on their way to the ganglion. This possibility was examined in the next experiments.

SECOND SERIES OF EXPERIMENTS. *Technique.* The operation was made as previously described, except that at the end of the dissection the bulla ossea was opened and its medial wall extirpated. The IX, X, XI and XII nerves were cut, and the internal carotid artery was severed between ligatures. The blood vessels under the postganglionic trunk were also ligated and divided. The perfusion was made in the usual manner; but in three experiments (VIII, IX, and X), immediately after starting the perfusion, the cat was killed by bleeding. The temperature in and around the ganglion was carefully maintained at 37 to 38 degrees C.

Results. The most striking result was the appearance in the perfusate of large amounts of A. Ch. in the absence of stimulation. In one case (VIII, 3/3/1937), the activity of the fluid collected without stimulation at the end of the experiment, when the rate of perfusion had become very slow, was greater than that during the preceding preganglionic stimulations. In fact, the activity of these control samples was estimated at 250 to 300γ A. Ch. per liter. In all four experiments of this series (VIII–XI) postganglionic stimulation was ineffective in liberating A. Ch.

In experiment XI (3/2/1937) the cat was kept alive. Figure 2 reproduces the responses of the leech to the samples of perfusate diluted 1:2.8 and to a solution of A. Ch. at 100γ per liter. Only one-half of the leech

Fig. 2

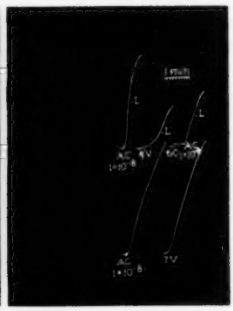

Fig. 3

Fig. 2. Responses of the leech to the perfusate and to a standard solution of A.Ch. (100γ per liter). Experiment XI (3/2/1937). The kink in the curve 13*D* was due to a wrinkle in the smoked paper. *V*, stimulation of the vagus. Dilution of the samples indicated in text.

Fig. 3. Responses of the leech to the perfusate obtained during a control period (6*C*); and two periods of vagus stimulation (4*V* and 7*V*) and a standard solution of A.Ch. (10γ per liter). Dilution of the perfusate 1:1.4. Experiment XV, (4/2/1937).

was assayed; according to the results of the tests the other half was less sensitive.

During the early part of the perfusion, the ganglion, even when it was not stimulated, yielded considerable A. Ch. As little physiological activity was to be expected on account of the deep narcosis, and as discharge from cut nerve fibers was impossible,—since the preganglionic nerve had not yet been divided,—the outpouring must have been attributable to the condition of the ganglion itself. As the experiment proceeded the spontaneous production diminished, and it was not visibly augmented when the preganglionic trunk was cut.

Neither stimulation of the postganglionic trunk (5 *A*, 11 *A*) nor of the vagus nerve (7 *V*) affected the output of A. Ch.; but preganglionic stimu-

lation augmented it to a marked degree (13 *D*), the concentration in the perfusate being estimated at over 250γ per liter. In the course of the observations it was noted that, while postganglionic stimulation during periods 5 and 7 caused maximal motor responses, preganglionic stimulation during period 13 was entirely ineffective. In order to locate the position of the block, strongly supermaximal stimuli were applied directly to the ganglion. As no response was evoked, it became clear that during the perfusion a block had developed somewhere between the ganglion cells and the postganglionic fibers. Thus no impulses could pass from the preganglionic fibers to the periphery, nor could any pass antidromically into the ganglion to effect a release of A. Ch.

When the ganglion was examined with a binocular microscope at the end of the perfusion, the capillaries in the distal part of the ganglion were seen to be filled with blood; that is, they had not been perfused and possibly an asphyxial block had developed. Accordingly the operative technique was modified.

THIRD SERIES OF EXPERIMENTS. *Technique.* The principal innovation in the method of preparation was not to cut the vagus nerve central to the ganglia, and thereby avoid the production of a stump which could retract and shut off the small venous capillaries in the neighborhood of the ganglia. Instead, after the bulla ossea had been opened, a silk loop was passed from the middle ear under the vagus nerve and the jugular vein, and then again under the postganglionic trunk, in a way so that when tied it interrupted the circulation through the internal carotid artery and the vessels accompanying the bundle of nerves, without interfering with the capillaries to the ganglion. Serial specimens of the perfusate were collected in amounts of 1 to 1.5 cc. (5–10 min.); but only selected samples were tested. The amounts of A. Ch. obtained in this series were small, and accordingly the frequency of stimulation was increased to 10 shocks per second.

Results. In experiment XII (3/3/1937) nine specimens were collected and five were tested on the same strip of leech. Sample 6 was collected before starting stimulation. In dilution of 1:1.72 it caused a slight contraction of the leech; sample 7 (postganglionic stimulation) in dilution of 1:2.8 produced a marked contraction; control sample 8 in dilution of 1:2.8 was entirely inactive; sample 9, obtained during stimulation of the vagus, was tested in dilution of 1:2.8 and found to be active, considerably more active than 6 (at 1:1.72 dilution), but less active than 7 at the same dilution. Finally sample 10 was collected during a preganglionic tetanus which caused maximal opening of the pupil and retraction of the nictitating membrane. It was tested in 1:7 dilution and found to be highly active.

Two facts were brought to light by this experiment: 1, that a postganglionic tetanization does not prevent a later transmission of preganglionic impulses through the ganglion, provided that the perfusion be made with

a conservative technique; and 2, that A. Ch. may be liberated not only by the antidromic conduction of impulses into the ganglion cells, but also by the passage of impulses through the nodosum ganglion of the vagus.

In the other three experiments of this series (XIII, XIV, and XV) stimulation of the vagus nerve in the neck also caused liberation of considerable amounts of A. Ch., but not with regularity. Thus in experiment XIII (3/16/1937) the vagus was stimulated during periods 7 and 16 and in both cases a large output of A. Ch. was recorded, while the control samples obtained immediately before and after stimulation were either inactive or only slightly active. In experiment XIV (3/17/1937) the vagus was stimulated during periods 5, 8, and 12. Samples 8 and 12 proved to be inactive, but sample 5 had the activity of A. Ch. $1:1 \times 10^8$, while control samples 4 and 7 were practically inactive. Again in experiment XV (4/2/1937) vagus sample 4 had a slight activity (fig. 3, 4 *V*); control sample 6 (fig. 3, 6 *C*) was inactive; but vagus sample 7 (fig. 3, 7 *V*) in dilution of 1:1.4 had an activity almost matching A. Ch. $1:1 \times 10^8$.

In view of the irregularity, the technique was again improved, this time in order to insure normal blood circulation through the ganglia until the very start of the perfusion.

FOURTH SERIES OF EXPERIMENTS. *Technique.* The operation was performed in a manner so that no large blood vessels had to be ligated until after the start of the perfusion. All the organs in the neck and mouth (glands, larynx, tongue) and the prevertebral and other muscles were successively extirpated after individual ligature of their blood vessels, often of capillary caliber (the operation was carried out under a binocular microscope). At the end nothing remained in the neck but the carotid arteries, the jugular vein, carotid glomus, vagosympathetic trunk, the two ganglia, and segments of the IX, XI, and XII nerves. After injection of a small amount of anticoagulant (Liquoid), a cannula was inserted into the common carotid artery and the perfusion started. A few seconds later, when the blood had been washed out, the main vessels were ligated and a collecting cannula was inserted into the jugulovertebral anastomotic vein. From four to five hours were needed for the operation, as over one hundred blood vessels had to be tied. In order to prevent cooling of the tissues, the room was kept at 34 degrees C. and the region of the field of operation was maintained at 37 to 38 degrees C., with the humidity at saturation by means of a vaporizer. Four of the experiments were so successful that histological sections of the perfused ganglia could not be distinguished from those from control ganglia which had not been perfused.

As in the previous group of experiments, the perfusate was collected in serial samples, and with but few exceptions all the samples were tested. The whole series was kept in the ice box at 0 degree C. until the end of the perfusion, as it had previously been shown that the storage would

not cause a detectable loss of activity (see fig. 9, 22 *C*). The series was then divided into two groups of consecutive samples, numbers 1 to 14, for example, making up one group and numbers 15 to 30 the other; and each group was tested on its own member of the paired leech muscle preparations. The slope of the contraction of the leech muscle was all that was necessary for a rough estimate of the A. Ch. content of the samples, and this could be determined after a few minutes of contact of the perfusate with the preparation. At the end of this period the leech was washed with running Locke's solution for several minutes. Then, after further washing with eserinized Locke's solution for 8 to 10 minutes, it was ready for the next test. Needless to say, contamination of the perfusate was prevented by using individual pipettes for each sample and by carefully avoiding contact of the fluid with the fingers of the experimenter (Dale and Feldberg, 1934).

Results. The fourth series included six experiments, five of which will be described and discussed individually. This is necessary because, despite all the precautions, the liberation of A. Ch. turned out not to be as regular a phenomenon as was anticipated from the descriptions of Feldberg and Gaddum, Feldberg and Vartiainen, and Brown and Feldberg. The liberation was even more irregular than had been suggested by the experiments of the three previous series.

Experiment XVI (4/7/1937). The control samples were collected at five minute intervals; but in stimulation periods the collection lasted for six minutes (five minutes of stimulation and one more minute without stimulation). All the samples but two (4 and 7, controls) were tested, all in 1:1.4 dilution. The sensitivity of the leech strips was assayed with A. Ch. at 5 and 10γ per liter.

Figure 4 reproduces the results of the tests. During the first 25 minutes of perfusion (samples 1 to 5) no A. Ch. was liberated, despite the section of the preganglionic trunk immediately before the start of the perfusion, and although there was one period of vagus stimulation (5). Evidently spontaneous liberation of A. Ch. can be reduced to a negligible amount or prevented altogether by the use of careful operative technique; and when it occurs, it cannot adequately be explained on the basis of mechanical injury of the preganglionic trunk.

Sample 6 obtained during stimulation of the vagus nerve began to contain traces of A. Ch., and a larger amount was obtained in the following sample, 8. Also a larger amount was obtained during the next stimulation of the vagus at 9 and in the following period 10. Sample 11, a control tested on the less sensitive half of the leech, was negative, as was also sample 12 from vagus stimulation; but the latter was succeeded by two samples, 13 and 14, which indicated a large amount of A. Ch. The next vagus stimulation at 15 came after the large output at 14, and it is difficult

to tell how much of the amount produced is in continuation of the latter and how much is new production. In this instance at least there was no delayed output, as the perfusate became negative at 17. At this point, preganglionic stimulation at 18 brought out large amounts of A. Ch. The production continued into 19, and after falling to a low point at 20, a markedly delayed production occurred during 21 and 22, more than 10 minutes after the end of the preganglionic tetanus.

According to the histological evidence, the experiment was a successful one. The ganglion and the unperfused control from the opposite side were stained according to Bodian and counterstained with acid fuchsin; and the two presented the same histological picture. Nevertheless there was a difference between the end of the experiment and the beginning,

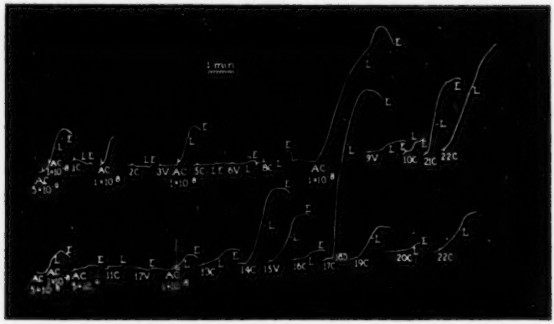

Fig. 4. Responses of the leech to the perfusate and to standard solutions of A.Ch. (5 and 10γ per liter). Experiment XVI (4/7/1937). Sample 22(C) was tested in both halves of the leech. Note that the solutions of A.Ch. are tested several times to detect changes in the sensitivity of the leech. Dilution of the perfusate 1:1.4.

for vagus stimulation evoked no A. Ch. at the beginning, not even a delayed production, while at the end a delayed liberation took place. Preganglionic stimulation differed from the vagus in that the output was larger and immediate as well as delayed.

Experiment XVII (4/8/1937). Twenty-four samples were collected and all were tested. Unless otherwise indicated, the collection periods were 5 minutes for the control samples and 6 minutes (5 minutes with stimulation and 1 minute without stimulation) for those with stimulation. The perfusion was carried out at a pressure of 60 cm. of water and the volumes of the samples were recorded. During the experiment the perfusion rate fell progressively to one-half of its original value. Although not as accurately measured, a falling off also occurred during all the other experiments, meaning no doubt that perfusion always occasioned some injury. No correlation between the appearance of A. Ch. and the rate

of perfusion could be established. All samples were tested in a 1:1.4 dilution, with leeches not very sensitive. The results of the tests are reproduced in figure 5 and compared with solutions of A. Ch. at 10, 50, and 100γ per liter. Table 1 summarizes the results of the experiment.

Only one preganglionic stimulation was tried (21). It brought forth A. Ch. promptly and in large amounts, the production outlasting the tetanus by 5 to 10 minutes. Antidromic tetanization during periods 2, 5, 8, and 14 caused only a delayed output of A. Ch. starting at least several minutes after the beginning of stimulation. Twice the production lasted only for a few minutes (samples 4 and 6), but in one case it lasted for 15 minutes (samples 15, 16, and 17). In one instance antidromic stimulation caused liberation of A. Ch. in two bursts, an initial one (sample 11) and a delayed one (sample 13). It was evident in every case that if the periods

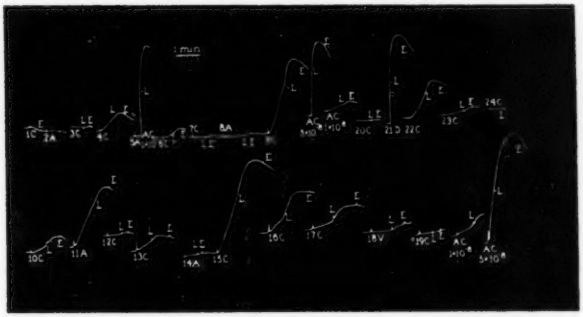

Fig. 5. Responses of the leech to the perfusate and to standard solutions of A.Ch. (10, 50 and 100γ per liter). Experiment XVII (4/8/1937). Dilution of the perfusate 1:1.4.

of collection of perfusate had been but slightly longer, the release of A. Ch. would have seemed to coincide in time with the stimulation.

Experiment XVIII (4/10/1937). In this experiment the effectiveness of preganglionic stimulation was checked by careful observation of the movements of the pupil and the nictitating membrane. Since the cat was under Dial narcosis, the pupil once opened never closed entirely, but the start of the opening and the start of the closing were easily detected. During every period of preganglionic stimulation the nictitating membrane was so retracted that it could scarcely be reached with a forceps, while during the control periods after it had been drawn out with a forceps it was found to be completely relaxed. The strength of stimulation necessary for threshold opening of the pupil was determined before starting the perfusion. During the experiment shocks from five to nine times this strength were used. From oscillographic studies it is known that even

the weakest of these strengths was maximal for the preganglionic trunk, and in fact the response of the pupil and the nictitating membrane were

TABLE 1

Experiment XVII (4/8/1937)

SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE	SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE
	minutes	*cc.*	*γ per liter*		*minutes*	*cc.*	*γ per liter*
1C	5	1.1	0	13C	5	0.8	10
2A	6	1.2	0	14A	6	0.9	2(?)
3C	5	0.9	0	15C	5	0.7	35
4C	5	0.9	20	16C	5	0.7	15
5A	6	1.1	0	17C	5	0.7	6
6C	5	0.8	10	18V	6	0.8	2(?)
7C	5	0.8		19C	5	0.7	0
8A	6	1.0	0	20C	6	0.8	0
9C	5	0.8	30	21D	6	0.8	100
10C	5	0.8	5	22C	7	0.9	20
11A	6	1.0	30	23C	7	0.7	5
12C	5	?	0	24C	10	1.1	0

TABLE 2

Experiment XVIII (4/10/1937)

SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE	SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE
	minutes	*cc.*	*γ per liter*		*minutes*	*cc.*	*γ per liter*
1C	4	1.5	2	19D	4	1.1	12
2D	2	0.7	10	20C	3	1.0	4
3C	3	1.1	4	21C	3	1.0	6
4C	3	0.9	2	22C	3	0.9	6
5C	3	1.0	0	23A	3	0.9	6
6D	2½	0.7	Less than 2	24C	3	0.8	6
7C	3	0.8	3	25C	3	0.8	3
8C	?	0.9	5	26D	2½	0.8	12
9C	3	0.8	3	27C	3	0.8	12
10D	3	0.7	10	28C	3	1.0	10
11C	4	1.2	2	29A	3	0.7	10
12C	4	1.2	Not tested	30C	?	1.0	12
13C	4	1.1	Not tested	31C	3	0.8	Less than 5
17C	3½	1.1	2	32D	4	0.8	10
18C	3	1.0	5	33C	3	0.7	5
				34C	4	0.8	Less than 5

always maximal. The frequency of stimulation was 10 shocks per second. The perfusion pressure was raised to 80 cm., so that a sufficient amount

of fluid could be collected in from 2 to 3 minutes. The amounts of fluid are indicated below. During period 14 (antidromic stimulation) escape of stimulus to the IX and X nerves caused reflex movements of the cat and displacement of the cannula with a resultant temporary interruption of the perfusion. The leech strips were of high sensitivity at the beginning of the tests, but the sensitivity as shown by frequent calibration with a standard solution of A. Ch. at 10γ per liter dropped progressively to about 50 per cent of its initial value. The results of the tests are reproduced in figure 6 and table 3, which contain a summary of the findings. The preganglionic tetanus lasted for one minute in periods 2, 6, 10, and 26, and for two minutes in periods 19 and 32. Antidromic stimulation lasted for three minutes in period 23 and for two minutes in period 29.

It is not necessary to emphasize that, because of the progressive loss in sensitivity of the leech, the concentrations of A. Ch. recorded in the

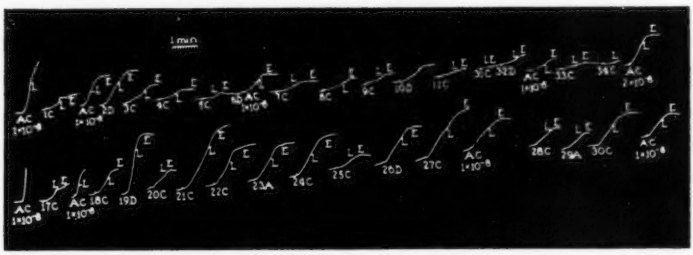

Fig. 6. Responses of the leech to the perfusate and to standard solutions of A.Ch. (10 and 20γ per liter). Experiment XVIII (4/10/1937). Note that the sensitivity of the leech progressively diminished but at the end of the experiment it still was almost 50 per cent of the initial one. Dilution of the perfusate 1:1.4.

table represent only a rough estimate of the amount present. They can, however, be safely considered as indicating the maximal amount of A. Ch. contained in the samples of perfusate.

The most remarkable feature of the observations was the small amount of A. Ch. released by preganglionic stimulation; for instance, in period 6 a tetanus made up of some 600 shocks caused maximal opening of the pupil and retraction of the nictitating membrane, but nevertheless failed to bring enough activity into the perfusate to cause visible movement in the leech muscle. This result was so unexpected that the preparation was immediately calibrated with A. Ch. at 10γ per liter to ascertain whether or not it was still active; and it was found that the sensitivity was only slightly less than during previous applications. If there was any A. Ch. in the sample, its concentration was less than 2γ per liter, i.e. about a hundredth part of the amount obtained in the early experiments. (In

the latter the rate of perfusion had been slower, but a lower frequency of stimulation compensated for the slow rate.) However, control samples 7 and 8 contained small but measurable amounts of A. Ch., indicating that there was some delayed output of the substance.

In the later periods of preganglionic stimulation the amount of A. Ch. obtained was somewhat larger, but still very small. Even when, after some 30 minutes of perfusion, A. Ch. began to appear in appreciable amounts in the control samples, preganglionic and postganglionic stimulations caused only small increases in the yield, and of the amounts produced a large fraction was released in the period following the peripheral re-

TABLE 3

Experiment XIX (4/13/1937)

SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE	SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE
	minutes	*cc.*	*γ per liter*		*minutes*	*cc.*	*γ per liter*
1C	3	2.4	Less than 5	16D	2	1.1	50
2D	2	?	15	17C	2	1.1	15
3D	2	0.8	More than 50	18V	2	Lost	
4C	2	1.1	15	19C	2	1.2	Less than 5
5C	2	1.0	10	20D	2	0.9	More than 50
6D	2	0.7	More than 50	21C	2	1.0	50
7C	2	1.1	20	22C	2	1.0	30
8C	2	1.0	Less than 5	23D	2	0.9	More than 50
9D	2	1.0	More than 50	24C	2	1.0	20
10C	2	1.2	12	25C	2	0.9	10
11C	2	0.8	10	26D	2	0.7	More than 50
12D	2	0.9	More than 50	27C	2	0.9	50
13C	3	1.4	15	28C	2	0.8	15
14C	2	0.9	Less than 5	29D	2	0.8	50
15C	3	1.4	10	30C	2	0.8	6

sponses. At the end of the experiment histological examination showed that this ganglion was in excellent condition.

Experiment XIX (4/13/1937) (fig. 7, table 3). The vaporizer was omitted in this experiment and in its stead the room was maintained at 37 degrees C. and at high humidity. Some difficulty was experienced, however, in keeping the tissues moist under these conditions. A chance observation in the course of the experiment indicated that A. Ch. appears promptly in the perfusing fluid. In period 2, which lasted two minutes, the perfusate of only the last half minute carried the products of activity resulting from preganglionic stimulation. Yet the perfusate was highly active. The appearance of the substance within 30 seconds supports Feldberg and Vartiainen's statement (1934, p. 111) that A. Ch. is removed

from the ganglion by the perfusion fluid in less than one minute after its liberation.

The durations of the preganglionic tetani (10 maximal shocks per second) were: 1 minute in periods 3, 9, 16, and 23; 1 minute and 40 seconds in period 20; and 2 minutes in periods 12, 26, and 29. The samples of perfusate were tested in 1:1.4 dilution.

As shown by the records in figure 7, preganglionic stimulation never failed to cause the release of considerable amounts of A. Ch. But the time of its appearance did not correspond closely with either the period of stimulation or the peripheral response. As usual, the peripheral response started immediately after commencement of stimulation and began to subside as soon as the stimulus was removed, relaxation of the muscles becoming complete, as far as it could be determined by direct observation, within less than a minute. But the release of A. Ch. continued for many

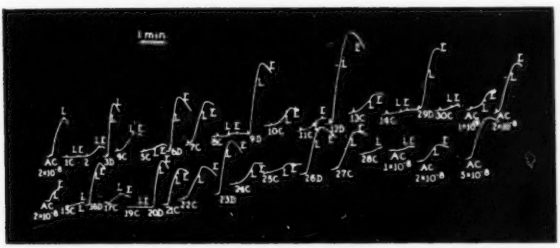

Fig. 7. Responses of the leech to the perfusate and to standard solutions of A Ch. (10, 20 and 50γ per liter). Experiment XIX (4/13/1937). The sensitivity of the leech muscle was low but remained constant. Dilution of the perfusate 1:1.4.

minutes after the end of stimulation. In some cases the liberation of A. Ch. was found to have ceased (insofar as the rather insensitive leech could detect) when the second control sample after stimulation was taken (4, 10, 17, 24, 30; 13 was not conclusive because one minute was lost between 12 and 13), but in other cases even the second control sample was highly active (7, 21, 22, 27). The prolonged output of A. Ch. could not have been due to slowed diffusion, first because as indicated above, the diffusion of A. Ch. is very rapid, and second because after stimulation period 29, when one should expect the diffusion to be slowest, no prolongation was present. Delayed liberation, which has been noted by Barsoum, Gaddum, and Khayyal (1934) and was seen in every experiment of this series, must mean delayed production.

Between control periods 14 and 15 the perfusion was stopped for 15 minutes, which may be the reason why sample 15 contained more A. Ch. than 14. The asphyxia of the ganglion caused a reduction of the periph-

eral response in period 16 and also a reduction in the liberation of A. Ch. in that period.

The rate of perfusion remained very nearly constant throughout the experiment. During most of the periods of preganglionic stimulation the perfusion rate was reduced, despite the large amount of A. Ch. which was formed.

When the ganglion was examined histologically, it was found to have been greatly damaged by the perfusion, probably because of the drying that had occurred in the course of its preparation. The ganglion itself and the tissues around it were invaded by a large number of white blood

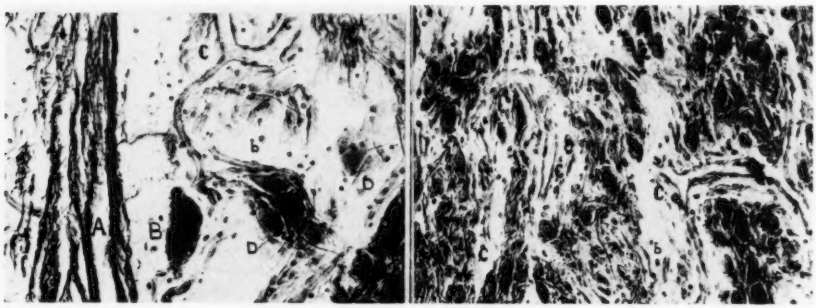

Fig. 8. Sections through the superior cervical sympathetic ganglion perfused in Experiment XIX (4/13/1937), Bodian's silver method, counterstain with acid fuchsin. Magnification 125×. The section on the left is through the periphery of the ganglion; it shows that the capsule of dense connective tissue, *A*, was separated from the ganglion by the edema. In the space thus created there are a capillary, *B*, blocked by a coagulum, an open capillary, *C*, with several branches, islands of ganglion cells, *D*, *D*, and a large number of white blood cells, *b*. The section on the right through the center of the ganglion shows the neuropil broken into small islands of cells by the edema around the capillaries, *C*, *C*, *C*. The ganglion cells are considerably retracted. Numerous white blood cells, *b*, are found in the edematous space.

cells. Edema had distended the dense capsule of connective tissue and created a large space between it and the ganglion proper, in which there were isolated capillaries and fragments of neuropil (fig. 8). The neuropil was generally fragmented and the cell bodies were shrunken and isolated into small patches. In every way the picture was in striking contrast with that of the ganglia in the preceding experiments in which the perfusions had been successful.

Experiment XX (4/16/1937) (fig. 9, table 4). In order more successfully to prevent drying of the tissues, during this experiment the humidity of the room was kept at saturation (37°). Owing to difficulty in making the insertion of the collecting cannula, the first samples were not obtained until 10 minutes after the start of the perfusion. After the eleventh sample,

the cat died, making observations of the responses no longer possible, but not causing interference with the ganglion which was on perfusion. The preganglionic tetanus (shocks more than six times the threshold strength for opening of the pupil, i.e., maximal at the rate of 10 per second) caused in periods 2 and 5 the usual maximal peripheral response; later, of course, no peripheral response could be obtained. The stimulation lasted $1\frac{1}{2}$ minutes in period 2, 2 minutes in 5 and 11, and $3\frac{1}{4}$ minutes in 20 and 26.

This experiment very nearly reproduced experiment XVIII in that at the beginning of the perfusion the amount of A. Ch. liberated was very

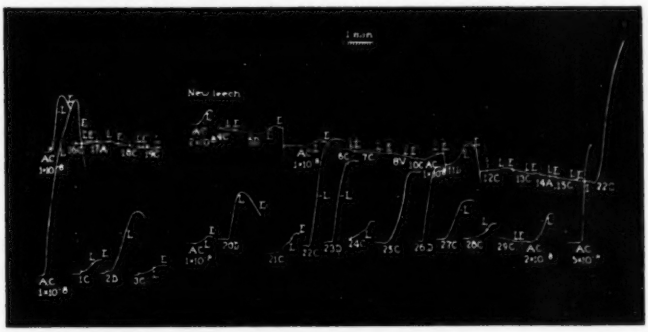

Fig. 9. Responses of the leeches to the perfusate and to standard solutions of A.Ch. (10, 20, and 50γ per liter). Experiment XX (4/16/1937). The responses in the left side were obtained with a leech of high sensitivity; those on the right side with a leech of less sensitivity. While the second leech was being prepared the perfusate was kept in the icebox at 0°C. Dilution of the perfusate 1:1.4. Sample 22 was tested on half of the upper series after further dilution 8:10 with eserinized Locke's solution, more than two hours after the test on the leech strip of the lower series. It showed no loss of activity.

small. The concentration in samples 2 and 5, collected while respectively about 900 and 1200 maximal shocks were being applied, certainly was no higher than 5γ per liter. Thus the amount of A. Ch. per maximal preganglionic volley was much smaller than the amount calculated by Feldberg and Vartiainen (1934). As the perfusion progressed, the A. Ch. production increased, both during periods of rest and of stimulation. It is to be noted that preganglionic tetani 20 and 23 caused liberation of A. Ch. in an initial and a delayed burst, and that after tetanus 26 the release of A. Ch. continued for 8 minutes after the end of the stimulation.

The histological analysis revealed that the greatest part of the ganglion was in apparently normal condition, but in the oral pole there were some distended capillaries surrounded by slightly edematous neuropil.

Discussion. The present study has revealed several significant facts:

1. In perfusions made with a technique likely to cause damage to the ganglia there appeared at the start of the perfusion in the absence of stimulation, a considerable output of A. Ch. (fig. 2, *1 c*). Careful technique considerably reduced or prevented this spontaneous release (figs. 4, 5).

2. In successful perfusions the liberation of A. Ch. by preganglionic stimulation was very small. For instance, the concentration of A. Ch. observed at the beginning of experiments XVIII and XX (fig. 6 and 9) was not greater than 5γ per liter; but when the perfusion was defective, the amounts of A. Ch. released were many times greater, up to 250γ per liter.

TABLE 4

Experiment XX (4/16/1937)

SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE	SAMPLE	DURATION OF COLLECTION	AMOUNT OF FLUID	APPROXIMATE CONCENTRATION OF ACETYLCHOLINE
	minutes	*cc.*	*γ per liter*		*minutes*	*cc.*	*γ per liter*
1C	3	1.3	1	16C	3	0.7	Less than 2
2D	2	0.7	5	17A	3	0.7	Less than 2
3C	3	1.1	1	18C	3	0.7	Less than 2
4C	3	1.0	Less than 5	19C	3	0.7	Less than 2
5D	3	0.9	5	20D	4	0.8	30
7C	3	0.9	Less than 5	21C	4	0.8	15
8V	3	0.8	Less than 5	22C	4	0.7	40
9C	3	0.8	Less than 5	23D	4	0.8	40
10C	3	0.8	Less than 5	24C	4	0.8	15
11D	3	0.8	15	25C	4	0.8	20
12C	3	0.8	5	26D	4	0.7	40
13C	3	0.8	Less than 5	27C	4	0.8	30
14A	3	0.8	Less than 5	28C	4	0.9	10
15C	3	0.7	Less than 5	29C	4	0.8	Less than 5

3. In the more complete experiments of the fourth series, in which histological analysis failed to reveal signs of damage to the ganglia, a definite relation between the output of A. Ch. and the duration of perfusion was established. At the start of the experiments the amounts of A. Ch. liberated were small, the minimal amount being produced in the second period of stimulation (figs. 5, *5 A*; 6, *6 D*; 9, *5 D*). The amounts increased during the perfusion and decreased again toward the end of the experiment. The increase in the release of A. Ch. was accompanied by a decrease in the rate of perfusion.

These facts raise the question as to whether the liberation of A. Ch. by nerve stimulation is a physiological process or, as recently Fleisch, Sibul, and Kaelin (1936) have concluded for motor nerves to striated muscles, a pathological process.

There can hardly be any doubt that the large outputs of A. Ch. observed

at the start of perfusion in the absence of stimulation, as well as similar outputs reported by Brown and Feldberg (1936 a, b), were attributable to injury of the tissues, for these amounts were prevented by improvements in the technique. Likewise the large outputs of A. Ch. observed after preganglionic stimulation in a number of the present experiments, as well as in the experiments reported by Feldberg and Gaddum (1934), Feldberg and Vartiainen (1934), Barsoum, Gaddum and Khayyal (1935), and Brown and Feldberg (1936 a, b), also in blood-perfused ganglia[2] must be considered as being pathological for the following reasons:

1. Physiological transmission through the ganglion takes place with release of much smaller amounts of A. Ch.

2. The amounts of A. Ch. liberated increase during the course of the experiment, as the rate of perfusion drops.

3. Ganglia releasing A. Ch. in large amounts are shown by histological analysis to have suffered heavy damage.

Taking the experiments as a whole, it must be concluded that the release of A. Ch. is favored by damage of the tissues. It also seems likely that some A. Ch. is released by dying cells, because in the experiments of the first and second series it was noticed that after a large spontaneous output of A. Ch. had taken place, further stimulations were ineffective in releasing the substance.

However, the fact that A. Ch. is released in any amount must indicate that the substance is found to some extent in the normal metabolism of the tissues of the ganglia. Whether or not the substance would diffuse through the surface of the cells under physiological conditions cannot be stated. At any rate, a diffusion such as this cannot take place to an extent greater than is observed in the early part of the most successful perfusion experiments (exps. XVIII and XX).

The metabolism of A. Ch. following nerve stimulation cannot be considered as a specific synaptic process, because A. Ch. may be released in considerable amounts by the passage of impulses through the nodosum ganglion of the vagus and by antidromic conduction of impulses into the ganglion cells. Furthermore, the liberation of A. Ch. usually outlasts the conduction of impulses and it may begin some time after transmission has ceased. Delayed production of A. Ch. cannot be doubted. The only question is whether there is any immediate production. Unfortunately, the technique of perfusion does not permit us to measure the time of liberation of A. Ch. within less than 30 seconds, while the synaptic

[2] In the opinion of the present author, satisfactory perfusion with blood of the isolated superior cervical and nodosum ganglia is not feasible. The operation would have to be performed as indicated in the fourth series of experiments, and the coagulation of the blood would have to be absolutely prevented. It appears that the prevention of coagulation is incompatible with a normal circulation and normal oxygenation of blood in the lungs; at least this was found to be the case with the anticoagulants employed (Chlorazol fast pink, Novirudin, Liquoid).

delay in sympathetic ganglia lasts for about 0.002 second (Brown, 1934; Eccles, 1933, 1936).

Examination of the records presented in figures 5, 6 and 9, which are those of the three best experiments, reveals that in general at the starts of the perfusions, preganglionic stimulation (fig. 6, *2 D*, *10 D*; fig. 9, *2 D*, *5 D*, *11 D*) caused an immediate output of A. Ch., while under similar conditions antidromic stimulation (fig. 5, *4 C*, *6 C*, *9 C*) seemed to cause only a delayed output. This might be considered as an indication that preganglionic stimulation causes release of A. Ch. during transmission, while ganglion cells liberate the substance after conduction. But the experiments also show that antidromic stimulation may cause an immediate output of A. Ch. (fig. 1, *5 A*, *7 A*; fig. 5, *11 A*), and that preganglionic stimulation may fail to cause an immediate output, while initiating a delayed one (fig. 6, *7 C*, *8 C*, *9 C*); or it may cause a delayed output (fig. 9, *22 C*) larger than the initial one (*20 D*). The similarity between records *20*, *21*, *22* and *23*, *24*, *25* in figure 9, and records *11*, *12* and *13* in figure 5 indicates that in both cases the same mechanism was being activated. Also, stimulation of the vagus may cause an immediate (fig. 3) or a delayed output (fig. 4), and in this case there can be no doubt that the A. Ch. is always released by the same elements.

Thus there is no evidence to show that preganglionic stimulation causes the production of A. Ch. by activating a mechanism fundamentally different from that activated by impulses started in the postganglionic trunk or in the vagus nerve; although it must be emphasized that preganglionic stimulation seems to cause an immediate output of A. Ch. more readily than any other stimulation. Thus far no satisfactory explanation has been found, either for the variability of the amount liberated or for the delayed output of the substance, but there are two facts which might lead to an understanding of the problem:

1. Postganglionic stimulation reaches only a fraction of the ganglion (the cells of origin of the carotid nerve), while preganglionic stimulation reaches, through the preganglionic arborizations, every cell of the ganglion. Evidently, if the concentration of A. Ch. released by preganglionic stimulation from ganglia in good condition scarcely reaches threshold for the leech, the concentration produced during postganglionic stimulation must remain below threshold.

2. Damaged ganglia release many times more A. Ch. than do ganglia in good condition. Therefore, if during the perfusion a few cells are damaged, they will release larger amounts of A. Ch. than the rest of the ganglion. In fact it is very likely that even in the best perfusions some of the A. Ch. liberated was released by damaged elements. Usually the first period of stimulation (figs. 5, *4 C*; 6, *2 D*; 9, *2 D*) caused a larger output than the second one (figs. 5, *6 D*; 6, *6 D*; 9, *5 D*); and no other explanation

for it can be found than the release of large amounts of the substance by a few cells damaged during the operation.

SUMMARY

1. The liberation of A. Ch. by the superior cervical sympathetic ganglion and the nodosum ganglion of the vagus has been studied with the technique of perfusion described by Kibjakow.

2. Ganglia prepared with a technique leading to damage of the cells as revealed by subsequent histological analysis yield A. Ch. to a perfusate without the intervention of stimulation; and stimulation of either the preganglionic or postganglionic trunks of the sympathetic or of the vagus nerve, causes the liberation of A. Ch. in large amounts.

3. Ganglia prepared in a way so that no damage is revealed on histological analysis do not yield A. Ch. spontaneously and the amounts released as the result of stimulation are very small. At the start of successful perfusions even perfusates obtained after tetanization of the preganglionic trunk with maximal shocks contain A. Ch. in amounts scarcely detectable by the leech preparation.

4. Release of A. Ch. regularly takes place some time after the transmission of impulses has ceased; whether there is any liberation preceding synaptic transmission is still an open question.

5. Since damage of the tissues favors the release of A. Ch. and perfusion of the ganglia always creates abnormal conditions, it cannot be stated whether physiologically any amount of A. Ch. diffuses out of the cells; but the evidence indicates the existence of an A. Ch. metabolism in the tissues of the ganglia.

6. The A. Ch. metabolism is not a process which is specific to the synaptic junctions.

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SPONTANEOUS MOTILITY OF THE PYLORIC SPHINCTER AND ADJACENT REGIONS OF THE GUT IN THE UNANESTHETIZED DOG

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Objections inherent in the usual methods of studying pyloric sphincter motility have made questionable the interpretations of many investigations. Results obtained from anesthetized animals are modified to an unknown extent by the drug action and those experiments involving exposure of the gut have the additional disturbing effects from various traumatic factors. Investigations based on gastric evacuation rate determinations (aspiration, duodenal fistula or Roentgen ray studies) are also susceptible to misinterpretation. At best, the information regarding the sphincter obtained by evacuation studies is entirely inferential. Evacuation depends on several factors in addition to sphincter behavior, which are incapable of accurate evaluation as the experiments are commonly performed.

Accurate determinations of pyloric sphincter tone and motility can be obtained *directly* by measuring the pressure and volume variations within the sphincter lumen. Such studies become particularily significant if performed with suitable precautions on unanesthetized animals as is possible when a balloon is introduced within the lumen. Additional information pertaining to the subject of gastric evacuation is obtained if the pressure and volume variations are studied simultaneously also in the regions adjacent to the sphincter.

We have made 250 direct determinations of tone and motility, simultaneously from the pyloric sphincter and 1.5 cm. at either side of it (in the pyloric antrum and duodenal bulb) and also in the distal duodenum in 10 dogs provided with gastric and duodenal cannulae. Tandem balloons (diagram 1) and water manometers were employed in a modification of the method of Thomas, Crider and Mogan. Special precautions were taken to minimize all trauma incident to the introduction or due to the presence of the balloons in the gut. The balloon system was soft and flexible, the tubes being made entirely of soft rubber and the balloons of thin condom rubber. This device was inserted practically without trauma by gentle traction on a thread permanently kept in the gut. A low pressure (2 cm.

water) was employed in the balloon-manometer system. The volume of air introduced was not sufficient to produce a positive pressure when the

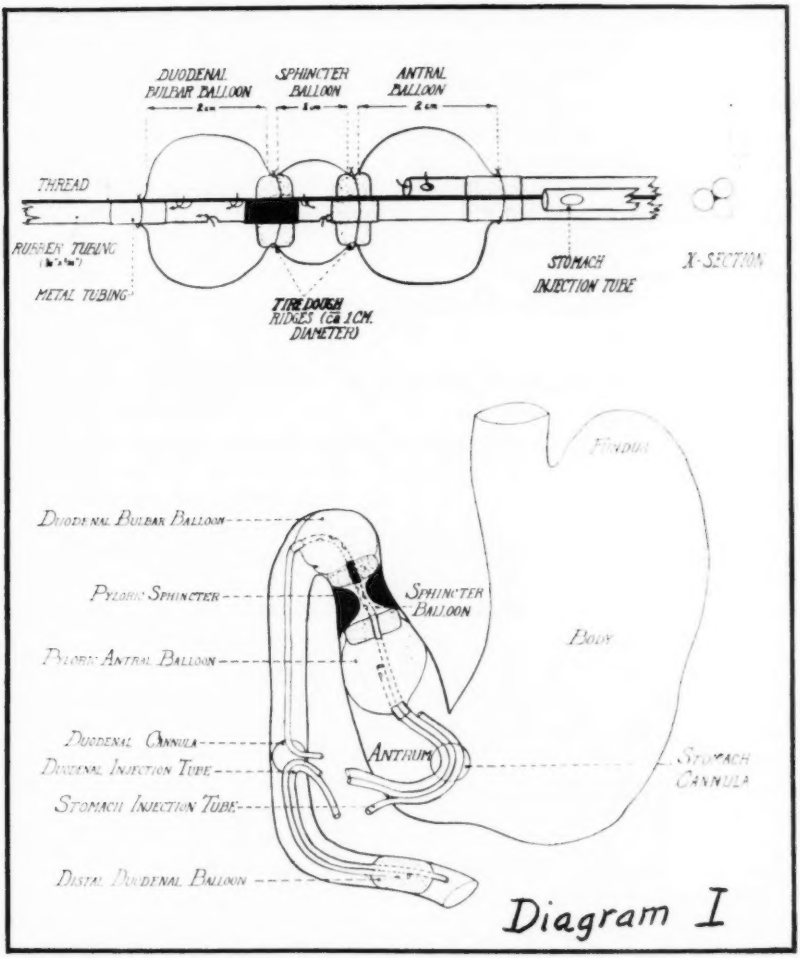
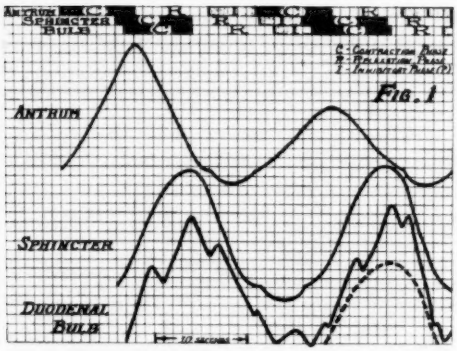
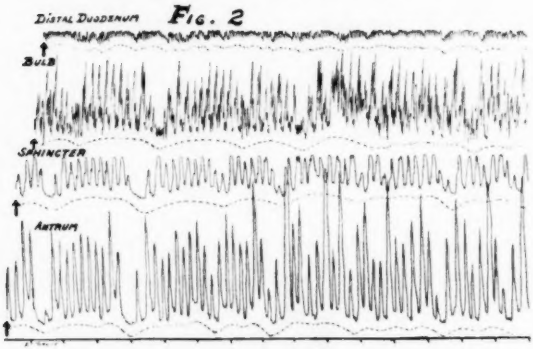


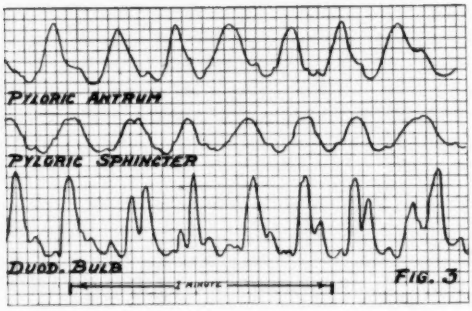
Diagram 1. Balloon system and the method of its insertion employed in studying motor activity of the pyloric sphincter and adjacent regions.

balloons were outside the gut lumen. Thus the factor of balloon elasticity was avoided.

Fasting animals. At the beginning of each of 250 experiments, food







Figs. 1-3

Fig. 1. Typical spontaneous motility of the pyloric antrum, sphincter and duodenal bulb of the normal dog during fasting and during gastric evacuation.

Fig. 2. Spontaneous motility of the pyloric antrum, sphincter, duodenal bulb and distal duodenum of the normal dog. Dotted lines indicate the tonus changes which in this experiment were more marked than usual.

Fig. 3. Spontaneous motility following chronic bilateral vagotomy.

had been withheld for 24 hours. The animals relaxed, completely at ease, on comfortable mattresses during each investigation.

Records made by this method showed that *ordinary peristaltic waves*, essentially the same as those occurring in other portions of the gut continually sweep over the antrum, sphincter and bulb at the rate of 2 to 5 per minute (fig. 1). There was no indication of the "contrary," "antagonistic," or "stop-cock-like" behavior in the sphincter which has previously been described. Practically every antral wave was followed by a sphincter and bulbar contraction which generally corresponded in amplitude, but on occasions showed individual variations. The bulbar contractions displayed more variations than occurred in other regions, there apparently being 3 to 6 locally initiated rhythmic movements superimposed on the fundamental contraction. A comparison of records made simultaneously from the bulb and the distal duodenum indicated that in the latter region the contractions were unrelated in sequence to those recorded from the bulb (fig. 2).

That the movements were peristaltic was indicated by the fact that a study of the time relations showed the contraction process of the antrum, sphincter and bulb to be progressive. Following a brief interval during which all three regions were simultaneously relaxed (fig. 1), a contraction occurred in the antrum. Since the sphincter at this time was relaxed, propulsion of antral contents could occur into the relaxed bulb. There was no unusual feature about the relaxation to agree with that implied by the terms "Law of Contrary Innervation" or "receptive relaxation" employed by Joseph and Meltzer and by Thomas.

Evidently the impulse to contract was conducted almost as rapidly to the bulb as to the sphincter or the sphincter had a longer latent period, for the contraction process occurred almost simultaneously in these two regions. Usually, however, the sphincter contraction slightly preceded the bulbar contraction and persisted during the contraction. This observation suggests that the sphincter acts to prevent regurgitation of duodenal contents rather than to interfere with gastric evacuation. Thus the sphincter functions not unlike the valves of the heart.

Since the balloon method measures volume and pressure changes within the gut lumen, it is particularly applicable to a study of the "wave of inhibition" which the Law of the Intestine states precedes the wave of peristaltic contraction. With the lever method, Alvarez and Mahoney could not distinguish relaxation of the intestine from passive distention produced by material pushed ahead of the progressing wave of contraction. Material forced ahead of such a wave would increase the pressure within a balloon while a wave of inhibition would lower the pressure. Records obtained by our method from the sphincter and adjacent regions usually showed a decrease in intralumen pressure preceding the contraction but

the decrease was not constant in occurrence and was small in magnitude, being much less than could be produced by certain experimental procedures. As recorded in our experiments, the "wave of inhibition" though demonstrable, appeared to be neither essential nor particularly important in peristalsis and unworthy of emphasis.

The tone of the antrum, sphincter and bulb (as indicated by a line joining the lowest point in each cycle) tended to remain constant but when changes occurred they were closely duplicated in all three regions (note dotted lines fig. 2). An entirely different pattern might be followed in other portions of the gut, e.g., the distal duodenum (fig. 2).

The antrum, sphincter and bulb continued to act as a unit (i.e., behaved in a similar manner) in the response to stimuli from the stomach or duodenum (results to be reported in detail elsewhere) or to emotions, disturbing sounds, etc. The effectiveness of these factors was greater on the proximal than on the distal structures as was evidenced by more complete inhibition and slower recovery in the antrum than in the bulb: the sphincter responses were intermediate. Of the four regions studied the inhibitory influences were least effective in the distal duodenum.

Passage of material through the sphincter region will be fundamentally dependent on the algebraic difference between antral pressure and bulbar pressure. The stomach will tend to evacuate when this quantity is positive; when the value is negative, duodenal regurgitation will be favored. The pyloric sphincter can influence the passage of material between stomach and bulb *a*, by its anatomical conformation; *b*, by the state of its contraction or relaxation. The factor *a* is essentially a constant, *b* is a variable. From the results obtained in this investigation, contraction of the sphincter was not a factor of great significance in the regulation of gastric evacuation. The magnitude of sphincter contraction was generally moderate (usually less than the antral contraction) and the duration was generally brief, being shorter than the relaxed period. Recalling the sequential relations in the antrum, sphincter and bulb previously described, the sphincter would normally appear to offer slight resistance to gastric evacuation and be moderately effective in preventing duodenal regurgitation.

During the process of gastric evacuation. Although the behavior of the antrum, sphincter and bulb is presented above as being characteristic of the fasting state, the descriptions apply with equal accuracy while gastric evacuation is in progress. In 125 experiments, substances were introduced into the stomach or duodenum of fasting dogs while motility was being recorded. Distilled water, tap water; hypotonic, isotonic and hypertonic sodium chloride solutions; sodium bicarbonate, glycerine, sugar and hydrochloric acid solutions; fatty acids, soaps, fat emulsions and other common substances were employed as test meals. Detailed ex-

amination of the records showed that the fundamental motility described above usually continued, although the rate and amplitude of contractions might be temporarily altered.

Following vagotomy. Analysis of 34 records of spontaneous motility made on 5 chronic animals following bilateral vagotomy showed no essential change in the motility of the antrum, sphincter and bulb either during fasting or during gastric evacuation. The spontaneous movements of these structures appeared fundamentally to be practically identical to those in the normal animal (fig. 3) and therefore were not dependent on vagus innervation. The amplitude of contraction in all three regions was moderately decreased following the operation and the peristaltic waves were sometimes slightly less frequent.

These results indicate that vagotomy alters the process of gastric evacuation (Meek and Herrin) by depressing the amplitude of antral contractions: changes in sphincter behavior not being significantly involved. Our observation of peristaltic waves passing into the duodenum after vagotomy should be contrasted with those of Ivy and Vloedman, who found no correlation between gastric and duodenal motility following surgical separation of these structures. Intrinsic, rather than vagal nervous connections appear to be essential for the passage of the gastric wave to the duodenum. In this connection, it should be recalled that vagotomy leads to marked degeneration of the interlacing network of the intrinsic nerves, only the local ganglion cells and their processes remain (Johnson).

Discussion. The gastric peristaltic wave passes on to the sphincter and the duodenum so the sphincter and bulb are in the relaxed or relaxing phase during antral contraction and are contracting during antral relaxation. Our observation of this phenomenon is supported by reports of several other investigators. As the result of fluoroscopic examination of man, Cole; Luckhardt, Phillips and Carlson; McClure, Reynolds and Schwartz; Hurst and Briggs and Klein reported that the sphincter opens during each antral contraction and the expulsion of a small amount of gastric contents occurs. The method employed, however, did not permit differentiation between dilatation of the sphincter due to sphincter inhibition from that produced by material forced into it from adjacent regions. Joseph and Meltzer, Payne and Poulton, Ivy and Vloedman, Quigley and Solomon made records simultaneously from balloons introduced into the stomach and duodenum and observed the passage of gastric peristaltic waves into the intestine. Freeman observed rhythmic peristaltic waves in the human antrum and sphincter during gastroscopic studies. In anesthetized or acutely operated dogs Wheelon and Thomas placed balloons in the antrum, sphincter and bulb and noted the passage of peristaltic waves in a manner agreeing closely with our observations on unanesthetized animals. However, in dogs not under drug influence Thomas and

Crider obtained somewhat different and more complex results. They concluded that the gastric influence on the duodenum was predominantly inhibitory. With the stomach empty they obtained irregular duodenal motility and the stomach was essentially quiescent. We have reason to believe that their results were distinctly modified by experimental methods. Alvarez and Mahoney emphasized a blocking of peristaltic waves at the sphincter but also described the "influence" of many gastric waves spreading onward to produce a "tone wave" in the duodenum upon which rhythmic contractions were superimposed.

Our observations show that the pyloric sphincter is essentially a relaxed structure, which contracts only at intervals, notably following arrival of a gastric peristaltic wave. Contrary to this is the general impression that the sphincter is closed at all times except when forced open by pressure developed from a neighboring region. The idea is well expressed by Cannon's description of peristaltic waves rhythmically pushing food against the sphincter, sometimes for half an hour without causing it to open. It is probably true that each antral peristaltic wave does not cause gastric evacuation even when the sphincter is not contracted and thus the impression may result, especially from x-ray studies, that a contracted sphincter is responsible (Haneborg, Klein). The failure of the stomach to empty may be due to 1: The antral waves which appear to be "powerful" may actually develop only moderate pressure and be insufficient for evacuation even through a relaxed sphincter. "Deep cutting antral waves" are produced with less effort if the intra lumen pressure is low than when more resistance is encountered. 2. The anatomical conformation of the sphincter probably prevents significant gastric emptying unless the antral propulsive force exceeds a certain value. This threshold value would be higher when the test meal contains solid material particularly when large particles are present. Thus the "sieve-action" of the pylorus may not necessitate a clamping shut of the sphincter. 3. Elevated pressures in the duodenum, such as develop, e.g., when the duodenum fails to relax or when duodenal contents fail to move onward, may also interfere with gastric evacuation. The tandem balloon method employed in our investigation definitely measures most of these factors.

SUMMARY

Employing the balloon method in unanesthetized dogs we have measured the tone and motility variations simultaneously in the pyloric antrum, sphincter and duodenal bulb. It was demonstrated that in the fasting state and also during gastric evacuation, frequent peristaltic waves sweep over these structures. The sequential relations show the sphincter and bulb are relaxed during antral contraction, thus gastric evacuation is favored. Contraction of the sphincter usually precedes and lasts through-

out bulbar contraction and thus interferes with regurgitation into the antrum which is relaxed during this interval. The "Law of the Intestine" has been demonstrated in this region but the "preceding wave of inhibition", though present, appears to be of but slight significance. A unity of response (tendency to behave as a unit) in these portions of the gut is emphasized in respect to the passage of peristaltic waves, tone changes and the effect of a variety of stimuli from the stomach or duodenum. The fundamental motility was not essentially modified by bilateral vagotomy.

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THE EFFECT OF OXYGEN DEFICIENCY ON THE SENSITIVITY OF RATS TO INSULIN

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The similarity of symptoms existing between insulin convulsions and anoxic convulsions (Olmsted and Logan, 1923) was the basis for investigating the anoxia theory of insulin convulsions. Holmes (1930) found that the oxygen consumption of brain tissues increased with increasing glucose concentration in the medium. Moreover, he demonstrated that animals which had been given insulin showed a lesser oxygen consumption by grey matter than did the brain of normal animals. Similar experiments were carried out by Wortis (1935), who confirmed Holmes' findings, and showed, also, that insulin diminished the oxygen consumption by brain tissue. Dameshek and Myerson (1935) observed in man that during severe hypoglycemia dextrose and oxygen consumption of the brain were materially reduced. These findings, recently confirmed by Himwich and Fazekas (1937), suggest strongly that in the production of insulin convulsions a state of anoxia is involved. In order to test this hypothesis the question was studied whether the exposure to low barometric pressure has a typical influence on the convulsions produced by insulin in rats.

METHOD. Prior to the insulin injections all rats were starved for 24 hours. Each rat was subjected to three experiments carried out in intervals of 5 days in which the same amount of insulin was injected intraperitoneally. In the second experiment the animal was placed for 2 hours in a low pressure chamber (at 460 mm. Hg) immediately after the injection. In the first and third experiment the animal remained under atmospheric conditions. The onset of the insulin convulsions was determined. Thus each animal was its own control.

Further studies were made on the effect of insulin on the blood sugar with and without oxygen deficiency, the former induced by exposing the animal to 460 mm. Hg for one hour. Each rat was again subjected to three experiments in intervals of 5 days. The blood was drawn from the heart, and the Folin and Malmros (1929) method was used.

RESULTS. In a preliminary set of experiments 20 rats were injected

[1] Preliminary report, Proc. Am. Physiol. Soc., 1936, p. 61.

with 3.5 to 4 units of insulin (Lilly) per kilo, and the same rats were 5 days later injected with the same amount of insulin and exposed for 30 minutes to a pressure of 460 mm. Hg. The rats were placed in the low pressure chamber after a time had elapsed equal to one-half the latent

TABLE 1

Latent period of convulsions in minutes

RAT	INSULIN	BAROMETRIC PRESSURE			RESULTS
		760	460	760	
	units per kgm.				
1	4.0	∞ *	341	∞	+
2	4.0	∞	94 died		+
3	4.0	∞	94 died		+
4	4.0	149	119	211	+
5	4.0	153	93 died		+
6	4.0	∞	116 died		+
7	4.0	∞	77 died		+
8	4.0	∞	94 died		+
9	4.0	∞	114 died		+
10	4.0	∞	226	228	±
11	4.0	186	180	∞	±
12	3.3	137	244	∞	±
13	3.5	143	∞	134	−
14	3.0	∞	189	∞	+
15	3.5	∞	179	∞	+
16	3.7	∞	69 died		+
17	3.4	∞	239 died		+
18	3.4	211	105 died		+
19	3.4	191	179	∞	+
20	3.4	170	120 died		+
21	3.4	∞	182	∞	+
22	4.0	∞	92 died		+
23	4.0	195	166 died		+
24	4.0	202	∞	231	−
25	4.0	∞	35 died		+
26	4.0	∞	119 died		+
27	4.0	∞	198	∞	+
28	4.0	∞	207 died		+
29	4.0	181	∞	227	−
30	4.0	206	179	237	+

* ∞ = no convulsions.

period of convulsions as determined in the first control. It was found that 17 rats showed convulsions in this series, which started earlier when the animals were subjected to the low pressure than in the control experiments carried out under ordinary atmospheric conditions before and after the low pressure experiments. Since, however, the differences in the latent

period of convulsions (time between injection of insulin and onset of convulsions) were not very great, it seemed advisable to extend the period of oxygen deficiency. In the main group of experiments 44 rats were injected with similar amounts of insulin. Fourteen of these rats did not show any convulsions. Therefore, their records are eliminated from the table. For the remaining 30 rats the latent period of insulin convulsions is given in minutes in table 1. When the latent period under low pressure is shortened a + is given under "Results"; when no change or a lengthening of the latent period occurred a ± and − respectively is indicated. It is evident from the table that the latent period of insulin convulsions was shortened in 24 cases (80 per cent). In three cases (10 per cent) (nos. 13, 24 and 29) the opposite result was obtained, whereas in three other cases (nos. 10, 11, 12), (10 per cent), the result was indecisive (±) because the differences were very small, or, as in no. 12, the latent period increased with repeated insulin injections so that the two controls did not check satisfactorily. Control experiments with rats not injected with insulin showed that the low pressure used did not produce any symptoms within 2 hours and convulsions did not occur even when the time of exposure was greatly lengthened.

The result, that in general the latent period of insulin convulsions is reduced when the rat is temporarily exposed to a diminished oxygen tension in the air, becomes more striking by studying the individual records. Table 1 shows that 16 of the 24 rats which showed a reduction in the latent period of convulsions died in the low pressure-insulin experiment, although glucose was immediately injected after the onset of convulsions. None of the rats so treated died when the insulin convulsions were produced by a similar amount of insulin under ordinary atmospheric pressure. In 5 other cases (nos. 1, 14, 15, 21, 27) the animals did not show insulin convulsions in the two control experiments which preceded and followed the low pressure-insulin experiment, but did so when the O_2-tension in the air was diminished. The results show clearly that the effects of insulin become greatly aggravated under conditions of a relative light anoxia which in itself has no apparent effects on the animals.

In a second series of experiments the influence of insulin on the blood sugar was studied under control and low pressure conditions. First it was found that the exposure of rats to 460 mm. Hg for one hour did not significantly alter the blood sugar. Prior to the experiment the mean was 77.6 mgm. per cent (standard error ±2.6). In the rats exposed to the low pressure chamber for one hour the mean was 81.5 mgm. per cent (standard error ±4.4). The results of these controls are in line with the experiments of Evans (1935), who found that even after a 3 hour exposure at a still lower pressure than used in our experiments no significant change in blood sugar occurred.

In the main group of experiments 19 rats were injected with insulin and the blood sugar determined one hour later. This experiment was repeated after five days while the animal was exposed to 460 mm. Hg for one hour, and 5 days later the control experiment (insulin at normal atmospheric pressure) was carried out again. The results of these experiments are given in table 2. They show that the decrease in blood sugar resulting from the insulin injection was slightly less at lowered barometric pressure than occurred in the controls carried out before and after in the same animals. We are not inclined to attach great weight to these differences since they are at the border line of statistical significance. They may indicate, however, that a given oxygen deficiency which does not produce any alteration in blood sugar in control animals may diminish the insulin effect on blood sugar by calling forth secretion of adrenaline. That this diminution in the blood sugar response to insulin is not due to a diminished sensitivity of the low pressure animals to insulin is clearly brought out by

TABLE 2

The decrease in blood sugar after insulin under normal and low barometric pressure

Series I.	Control	
	Mean decrease in blood sugar	37.7
	Standard error	±1.68
Series II.	Four hundred and sixty millimeter barometric pressure for 1 hour	
	Mean decrease in blood sugar	23.8
	Standard error	±1.51
Series III.	Control	
	Mean decrease in blood sugar	30.2
	Standard error	±1.30

the shortened latent period of the convulsions and the greater fatality. At any rate, the experiments show that this increased insulin sensitivity does not involve a greater decrease in blood sugar.

Two conclusions may be drawn from these experiments.

1. Insulin convulsions are more rapidly precipitated in rats when these animals are subjected temporarily to a period of oxygen deficiency which in itself is apparently without any harmful effect.

2. If the blood sugar determinations obtained one hour after insulin are a fair indicator of the later course of the blood sugar it is probable that at low oxygen tension the insulin convulsions are precipitated at the same or an even slightly higher blood sugar level than is observed in rats subjected to the same amount of insulin at atmospheric pressure.[2]

Since the low pressure employed in these experiments does neither alter

[2] Blood sugar determinations during insulin convulsions are unsatisfactory because of the influence the degree and duration of convulsions have on the blood sugar level.

the blood sugar nor induce any signs of anoxia in the control experiments (without insulin) and since it causes the insulin convulsions to occur earlier and very frequently in a more severe form (greater fatality) it may be concluded that insulin and oxygen deficiency have a potentiating effect on each other. This is easily understandable if we, on the basis of the observations of Holmes, Wortis, Dameshek and Myerson reported above, assume that hypoglycemia produces a state of relative anoxia in the brain. If this is the case the sensitivity of a hypoglycemic animal to oxygen deficiency must be greater than that of control animals with a normal blood sugar. This conclusion is supported by the work of Moldavsky and Gellhorn (1937), who showed that the effect of the inhalation of gas mixtures low in oxygen on the blood pressure was greatly augmented during hypoglycemia. Further, the behavior of the animals during insulin convulsions under atmospheric pressure and in the low pressure chamber favors this interpretation.

Under atmospheric pressure, after a convulsive dose of insulin has been administered, the rat begins to show signs of depression and as this depression becomes more marked, it drags its tail and has a tendency to keep, or press, its body on the bottom of the cage. Later, it spasmodically jerks in various directions and finally twists and turns over and over.

The animals that convulsed in the low pressure chamber after insulin showed the same symptoms except the twisting and turning. These animals just before convulsing jump up and down several times and then fall on their side. Only once did an animal in the low pressure chamber start to twist but its final movements consisted of a high leap and fall after which it remained quiet. This picture in the low pressure chamber is practically the same as when normal animals are subjected to very severe anoxia. Thus it appears as if the insulin injected animals were suffering from a relative anoxia which is greatly aggravated when the animals are exposed to a reduced oxygen pressure, although the latter is entirely harmless to noninsulinized control animals.

SUMMARY

The effect of oxygen deficiency (reduction of the barometric pressure to 460 mm. Hg) was studied on the production of insulin convulsions and hypoglycemia in rats. The results are:

1. The insulin convulsions show a greater severity and a shortened latent period under low pressure.

2. The combination of insulin and low pressure does not cause the blood sugar to fall below the level observed in the same animals when the rats remain under atmospheric conditions after the injection of insulin.

3. The clinical picture of convulsions shown by the rats in the low pressure chamber resembles that of rats suffering from extreme degrees of anoxia.

4. The low pressure (460 mm. Hg) used in these experiments is without any apparent effect on noninsulinized rats even when the time of exposure is many times greater than that employed in our experiments.

These facts seem to indicate that low blood sugar interferes with the oxygenation of the central nervous system. Therefore a mild degree of oxygen deficiency under conditions of low blood sugar leads to symptoms which are similar to those obtained at normal blood sugar level only under conditions of extreme anoxia.

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THE IMMATURE RAT UTERUS AS AN ASSAY END-POINT FOR GONADOTROPIC SUBSTANCES[1]

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To determine the effect of various treatments upon the gonadotropic content of the hypophysis of the rat, mouse and other laboratory animals, the method of choice has usually consisted of implanting entire glands, or multiples thereof, into immature female rats or mice and employing increase in ovary weight as the end-point. There are serious objections to these methods: *a.* As Emery (1) has shown, some implants become vascularized and continue to elaborate hormones, others are absorbed completely without "taking," while still others may become encapsulated by connective tissue. Kylin (2) likewise introduced evidence to show that small pieces of pituitary gland can become vascularized and remain functional for months. *b.* The implantation methods have the further disadvantage of being only roughly quantitative, that is, only the potency of one or more whole glands can be conveniently measured. Use of the implant method in this laboratory has led to great variation in results. *c.* The use of the ovarian end-point requires relatively large amounts of material, usually one or more glands, to produce a positive response. When statistically significant results are desired, on large numbers of test animals, the number of donor glands required becomes almost prohibitive.

In looking beyond the ovarian weight changes as the end-point for assaying materials for gonadotropic content, we had the following objectives in mind: *a,* to find an end-point that would be sensitive to small amounts of material and which would permit the use of a sufficiently large number of animals to be statistically significant without exhausting a limited supply of material; and *b,* to use a method other than implantation that would result in a more uniform and reliable response in the recipient animal.

Most workers who have had occasion to use the immature ovary as an assay end-point have observed a collateral uterine stimulation. A. E. Severinghaus (3), Emery, Bash and Lewis (4), Davy (5), and others have

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pointed out the phenomenon of uterine enlargement, but usually only in connection with enlarged ovaries, and in general have not considered it constant and predictable enough to be useful as an end-point. In 1928, Siddall (6, 7) used the combined uterine and ovarian weight of the immature mouse as an end-point for a pregnancy test, and suggested the method for qualitative assay of pituitary and placental extracts. Cahen and Ardoint (8) published a note in 1936 on the use of uterine weights as a method for the assay of pituitary extracts. They did not state whether the uteri were weighed with or without the intra-uterine fluid intact. In a previous study on the effect of estrin treatment upon the gonadotropic potency of the pituitary of the castrated female rat (to be published), the present authors found that the uterus of the immature rat served as a very sensitive and reliable end-point. The method consisted of determining the smallest amount of pituitary suspension which caused a definite, grossly-visible, enlargement of the uterus. We found this amount to be about $\frac{1}{6}$ of that required to produce minimal ovarian weight increase. Shortly after the completion of this experiment, Levin and Tyndale (9) announced the use of increase in the weight of the immature mouse uterus in the assay of castrate and menopause urine extracts. They tentatively defined the mouse unit as the minimal amount, which when given twice daily for three days to five mice, would produce a 100 per cent increase in average uterine weight (fluid removed before weighing). An error of no more than 25 to 30 per cent was claimed. Owen and Prince (10) have published a chart showing stages in the changes in macroscopic size of the uterus and ovaries, as a guide for quantitative assay of pregnancy urine and certain pathological urines, but they make no mention of the quantitative relationships of the various stages, or reactions.

Thomsen and Pedersen-Bjergaard (15) have recently reported a gonadotropic assay method in which changes in vaginal histology are utilized. Their rat unit is defined as the smallest quantity of the material which, when injected over a three day period into immature rats, will produce full cornification of the vagina, as determined by histological section. These authors have standardized the reaction of doses less than 1 R.U. and hence are able to assay small amounts with considerable accuracy.

The present study was undertaken to make a comparison, on a statistical basis, of the following gonadotropic assay end-points: minimal uterine enlargement, uterine weight increase, vaginal opening, vaginal weight increase, and minimal ovary weight increase. For this purpose, the pituitary gland of mature female rats, castrated for a period of 20 days, was chosen as the source of gonadotropic material for the following reasons: *a*, it is relatively potent; *b*, lends itself to comparison with normal and treated rat pituitaries, and *c*, serves as a standard which can readily be reproduced.

METHODS. Extraction or desiccation of the pituitary glands as a preliminary treatment to injection might conceivably overcome some of the disadvantages of the implant method. However, until the chemical nature of gonadotropic substances is more completely understood, it was felt that the use of fresh, untreated materials would eliminate the introduction of extraneous factors which might result in alteration of potency and loss of material. In order to exclude the undesirable features of the implant method, and yet use fresh materials, we adopted a suspension method suggested to us by Doctors Meyer and Hertz of the Zoology Department. We have found it elicits a more uniform response as judged by ovarian weight increase, as well as by other criteria to be described, and has the added advantage that small and exact fractions of the gland can be assayed.

Fifty-seven mature female rats of the highly-inbred Sprague-Dawley strain, ranging from 19 to 30 weeks in age, were ovariectomized in groups of nine to fourteen on five different days. After a period of 20 days, the rats of each group were killed by rapid decapitation, the whole pituitaries aseptically removed, and placed in a 15 cc. sterile, ice-cooled graduated centrifuge tube. The pooled glands of each group were thoroughly macerated by means of a glass rod ground to fit the bottom of the tube. Sterile distilled water was added in a quantity calculated to give a resulting suspension of definite concentration. The suspension was then broken into very fine particles and thoroughly mixed by being alternately drawn into and ejected from a small syringe fitted with a fine gauge needle. This treatment lysed practically all of the cells, so that microscopically only cellular debris and occasional clumps of connective tissue fibers were seen. The possibility that any cells would grow and continue to elaborate hormones in the tissue of the recipient was thus virtually eliminated. From this stock suspension graded dilutions were made to represent *total* dosages ranging from 0.25 mgm. to 8.0 mgm., when administered in nine subcutaneous injections of 0.5 cc. each, twice daily, over a period of $4\frac{1}{2}$ days.

Two hundred and twenty-eight female rats from the Sprague-Dawley colony, exactly 21 days of age and ranging from 34 to 39 grams in weight at the beginning of the injection period, were employed as test animals. They were divided into five series of 42 to 48 rats each, corresponding to the five groups of castrate donors. The assay was conducted in five series, with 3 rats (2 or 4 in a few cases) on each dose level in each series, representing a total of 15 animals on each of the 15 dose levels. In this way we hoped to be able to detect any slight differences in potency among the five pooled hypophyseal suspensions, as well as to be able to observe the individual variations occurring on any dose *within* a given series; in other words, to study the variability among the five groups of glands, as well as the variability of response on the part of the test animals.

Injections were begun the evening of the day of donor autopsy and continued morning and evening until a total of 9 injections had been given. Great care was taken to keep leaks at site of injection at a minimum. The suspensions were stored at 4°C. between injections. The rats were autopsied the morning after the last injection, when they were 26 days of age. The degree of gross uterine enlargement was noted, with the uterus in situ, and graded according to the following scale (see figs. 1 to 4): infantile; greater than infantile but less than one plus; one plus; two plus; and three plus. The uterus, ovaries, and vagina were removed and dissected free of fat and connective tissue. The uterus was separated from the vagina by cutting through the cervix and from the oviducts at their

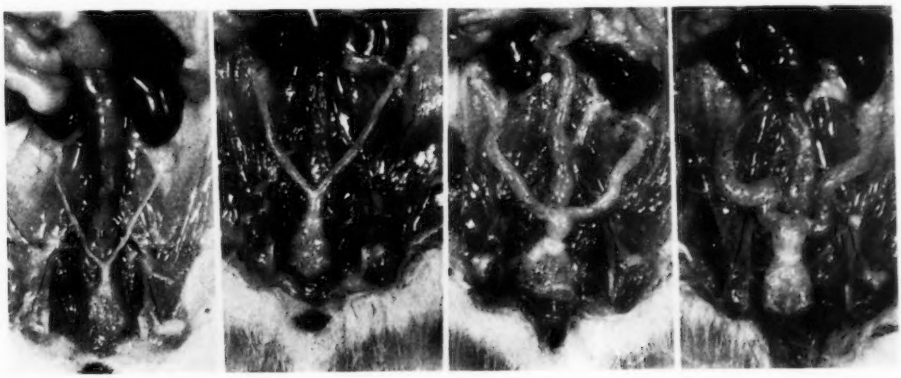

Fig. 1 Fig. 2 Fig. 3 Fig. 4

Fig. 1. Infantile uterus.
Fig. 2. One plus enlargement.
Fig. 3. Two plus enlargement.
Fig. 4. Three plus enlargement. Surrounding fat has been removed. Approximately natural size.

junction with the uterine horns. Each organ was weighed separately, the uterus being weighed before and after the fluid had been gently pressed out on moistened blotting paper, according to the technique of Levin and Tyndale (11).

Results. The results will be presented first considering the five series as a whole, and then the results of each series will be analyzed separately.

Vaginal opening occurred first at the 1.75 mgm. level in 3 of 15 animals. At 2.0 mgm. 8 of 15 were open; at 3.0 mgm. 12 of 15; yet in no group above this level were all vaginas open. Even at the highest level, 8.0 mgm., 4 of 15 vaginas failed to open. (See table 1.)

Vaginal weight. The mean and extreme vaginal weights are shown in

figure 5b. The control average was 16.1 mgm. with range from 13.3 mgm. to 19.8 mgm. The first significant increase above this level occurred in the 0.75 mgm. group, the mean being 21.1 mgm., or 32 per cent increase. (See table 1.) The curve of the means rises rapidly to a maximum at the 2.0 mgm. dose, and recedes very slightly in the higher dose levels. The close correlation with uterine weight (minus fluid) is seen in figure 5 and table 1. The range of variation is smaller than in the case of the uterus, but the average weights are correspondingly less, so that the relative variation is about the same in both cases.

Gross uterine enlargement above infantile size was absent in all cases in the 0.25 mgm. and 0.50 mgm. groups. The first definite visible enlargement occurred in the 0.75 mgm. dose group in 6 out of 15 rats. At 1.00 mgm. 13 of 15 responded; at 1.25 mgm., 13 of 15; and above this dose

TABLE 1

Summary of vaginal, uterine, and ovarian response

The total number of positive responses, based on definitions given in the text, are given for each dose. There were 15 animals on each dose.

END-POINT	DOSE IN MILLIGRAMS														
	Control	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Vaginal opening	0	0	0	0	6	0	0	3	8	12	12	13	13	14	11
Vaginal weight	0	5	3	9	12	13	14	15	15	15	15	15	15	15	15
Uterus enlargement	0	0	0	6	12	12	13	15	15	15	15	15	15	15	15
Uterus weight	0	0	0	6	10	12	13	15	15	15	15	15	15	15	15
Ovary weight	0	0	0	0	0	0	0	0	0	0	0	1	3	2	5
Average weights															
Vaginal weight	16.1	18.0	16.9	21.1	25.6	32.0	42.4	42.3	45.5	42.0	42.0	43.8	44.5	38.8	36.8
Uterine weight	18.9	18.6	20.2	27.3	37.1	45.6	68.4	72.8	83.6	80.0	71.8	75.2	78.0	59.2	67.0
Ovarian weight	12.1	12.3	11.7	10.2	10.5	11.0	11.1	10.7	11.6	11.1	13.9	13.7	15.8	15.0	17.0

all uteri exhibited a positive visible enlargement. Table 2 shows the frequency distribution of the various grades of enlargement at all dose levels. The response reaches a maximum between 1.75 mgm. and 4.0 mgm. and gradually decreases in the highest doses. Table 4 illustrates the relative accuracy of gross judgment of uterine enlargement as compared with the use of uterine weights. The similarity of the distributions shown in tables 2 and 3 is quite striking (the degree of enlargement was graded before weighing in all cases).

Uterine weight. (See fig. 5 and tables 1 and 3.) The first weight increase occurred at the 0.75 mgm. dose, with 6 of 15 uteri exceeding control range, representing a mean increase of 50 per cent above control average. At the 1.00 mgm. dose, 10 of 15 uteri were above the control weight range, a mean increase of 100 per cent. The curve rapidly rises to a maximum

at 2.0 mgm. and gradually recedes as the dose increases. To test the reliability of the differences between the successive means on the ascending part of the curve, Fisher's (12) method of t was used. The probability that a given difference is due to chance was calculated. If the probability was less than one or two chances in one hundred, the difference was considered as quite significant. On this basis, the mean uterine weight of

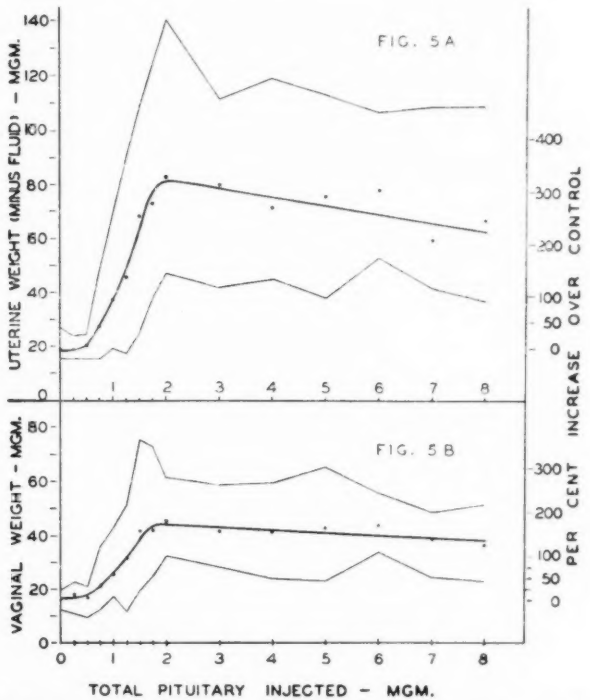

Fig. 5 A. Mean and extreme uterine weights of all series combined. Each point represents the mean of 15 uterine weights.

5 B. Mean and extreme vaginal weights of all series combined. Each point represents the mean of 15 vaginal weights.

the 0.75 mgm. dose group significantly exceeded the means of the controls, the 0.25 mgm., and 0.50 mgm. groups; the mean of the 1.00 mgm. group significantly exceeded the mean of the 0.75 mgm. group; the difference between the 1.25 mgm. and 1.00 mgm. groups was not significant; the mean of the 1.50 mgm. group was significantly greater than either the 1.00 mgm. or 1.25 mgm. means. The differences between the remaining successive means were not significant.

Ovary weight. Table 1 gives the mean ovary weights for all dose levels, and indicates the number of rats in each dose group the ovaries of which exceeded the control range (maximum control weight was 17.7 mgm.). Fifteen control weights from rats of the same age are added from a previous study. The mean weights are within normal limits up to the 0.75 mgm. level. At the 0.75 mgm. and 1.00 mgm. doses, the mean ovary weight

TABLE 2

Frequency distribution of the stages of uterine enlargement

DEGREE OF UTERINE ENLARGEMENT	DOSE IN MILLIGRAMS														
	Control	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.0	3.0	4.0	5.0	6.0	7.0	8.0
Infantile	18	15	15	9	3	3	2								
> Inf. < One plus				4	5	4	1	4			1				1
One plus				2	7	8	8	6	8	10	13	11	14	14	13
Two plus							4	1	4	3		2	1	1	1
Three plus								4	3	2	1	2			

TABLE 3

Frequency distribution of uterine weight (minus fluid)

UTERINE WEIGHT INTERVALS (MGM.)	DOSE IN MILLIGRAMS														
	Control	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.0	3.0	4.0	5.0	6.0	7.0	8.0
15.0– 26.8	18	15	15	9	5	3	2								
26.9– 34.9				3	1	1									
35.0– 44.9				1	6	3	1	3		1		2		1	1
45.0– 54.9				2	1	5	1	3	3	2	3	1	1	6	3
55.0– 64.9							2	1	3	1	5	4	2	6	5
65.0– 74.9					2	2	2	3		1	2		2		1
75.0– 84.9							3		3	3	2		4	1	2
85.0– 94.9						1	2		1	3		2	5		2
95.0–104.9							1	1	1	2	1	5			
105.0–114.9							1	2	1	1	1	1	1	1	1
115.0–124.9								2	2	1	1				
125.0–134.9															
135.0–144.9									1						

shows a slight, but statistically significant, decrease below control level. At the 6.0 mgm. level the first statistically significant increase occurred. This level is taken as the unit of minimal ovary weight increase for the five series taken as a whole.

Analysis of results of each series. Table 5 summarizes the results of the individual series. The number of donor animals, their mean age and body weight at autopsy, and the mean pituitary weights are given. The re-

maining columns indicate the doses of the pituitary suspension in milligrams which elicited unit response in each of the end-points investigated. The units are defined below.

Vaginal opening. The unit is defined as the minimal amount, when administered as described above, which caused vaginal opening in at least

TABLE 4

Frequency distribution diagram, illustrating the relative accuracy of judgment of gross uterine enlargement as compared to uterine weights, minus fluid

UTERINE WEIGHT INTERVALS (MGM.)	DEGREES OF UTERINE ENLARGEMENT				
	Infantile	>Inf. <one plus	One plus	Two plus	Three plus
15.0– 26.8	65	3			
26.9– 34.9		5			
35.0– 44.9		9	9		
45.0– 54.9		3	28		
55.0– 64.9			28		
65.0– 74.9			15		
75.0– 84.9			16	2	
85.0– 94.9			14	1	2
95.0–104.9			3	7	1
105.0–114.9			1	5	3
115.0–124.9				2	4
125.0–134.9					
135.0–144.9					1

TABLE 5

Summary of the results of the individual series

For unit definitions see text

SERIES	NUMBER OF DONORS	MEAN DONOR AGE (WEEKS)	MEAN DONOR WEIGHT (GRAMS)	MEAN PITUITARY WEIGHT (MGM.)	UNIT DOSES IN MILLIGRAMS				
					Vaginal opening	Vaginal weight	Uterine enlargement	Uterine weight	Ovary weight
I	11	28	256	14.0	3.00	1.00	1.00	1.00	7.0
II	11	27	253	13.2	1.75	0.75	0.75	0.75	6.0
III	12	24	268	13.4	2.00	0.75	0.75	0.75	6.0
IV	9	23	270	13.3	2.00	0.75	0.75	0.75	8.0
V	14	24	260	13.0	1.75	0.75	0.75	0.75	5.0

one of three test animals. From table 5 it is seen that in series II and V the minimal response occurred at 1.75 mgm.; in series III and IV, at 2.0 mgm.; and in series I, at the 3.0 mgm. dose. Vaginal opening as a gonadotropic end-point has been far less satisfactory, in our experience, than any of the others employed, for the following reasons: *a*, it is quite variable; *b*, even at the highest dose level employed, some vaginas failed to open;

c, many of the unopened vaginas weighed as much as, or even more than, many of the vaginas which had established the orifice, and *d*, it is less sensitive than uterine or vaginal weight end-points.

Vaginal weight. The control average weight was 16.1 mgm. with a maximum of 19.8 mgm. The unit may conveniently be defined in two ways, both giving essentially the same result; namely, as the minimal amount of suspension which produces a mean vaginal weight *a*, 25 to 30 per cent above control average, or *b*, which exceeds the highest control weight, i.e., above 19.8 mgm. On the basis of these definitions, the unit dose for series I was 1.0 mgm., and for all the others, 0.75 mgm., corresponding to the results on uterine enlargement and uterine weight. It is obvious that this end-point is more uniform, more sensitive, and more reliable than vaginal opening. However, since *a*, a very high degree of correlation exists between uterine and vaginal weights, and *b*, the uterus is more easily removed and prepared for weighing than the vagina, and *c*, the uterine weight curve (see fig. 5) is steeper at the minimal level, thereby permitting more accurate localization of the unit dose, it is concluded that the use of vaginal weights offers no improvement over uterine weight as an assay end-point.

Vaginal histology. In one series of animals representative vaginas were sectioned and measurements made. It was found that slight histological changes occurred at the 0.5 mgm. dose, indicating that vaginal histology might serve as a somewhat more sensitive end point than uterine weight. On the whole, vaginal histology substantiated the uterine weight changes. As the dose was increased, there was a progressive change in epithelial thickness, cell types, and number of cell layers. For example, in the control animals the epithelium averaged 0.018 mm. in thickness, consisting of 3 layers. At the 0.5 mgm. dose level, the thickness averaged 0.020 mm., cuboidal cells predominated, and there were 3 to 5 cell layers. At 1.0 mgm., the epithelium was 0.045 mm. thick, the cell type, statified squamous, and the cell layers numbered from 6 to 10. At 2.0 mgm. the thickness was about 0.08 mm., the superficial cells were cornified, and there were more than 10 cell layers. Comparison of our findings with the excellent photographs and text descriptions of Thomsen and Pedersen-Bjergaard (15) indicate that 2 mgm. of our 20-day castrate pituitary suspension are approximately equivalent to one of their rat units (assuming no marked strain difference in reactivity), while 1 mgm. roughly corresponds to the stage which they designate as $\frac{1}{2}$ to $\frac{2}{3}$ rat unit, and 0.5 mgm. is approximately equivalent to the reaction they obtain with $\frac{1}{5}$ R.U. We are thus able to confirm the findings of these authors. However, it seems quite evident that the uterine weight method is not only much simpler than, but practically as sensitive as, a method based on vaginal histology.

Gross uterine enlargement. The unit is defined as the smallest amount

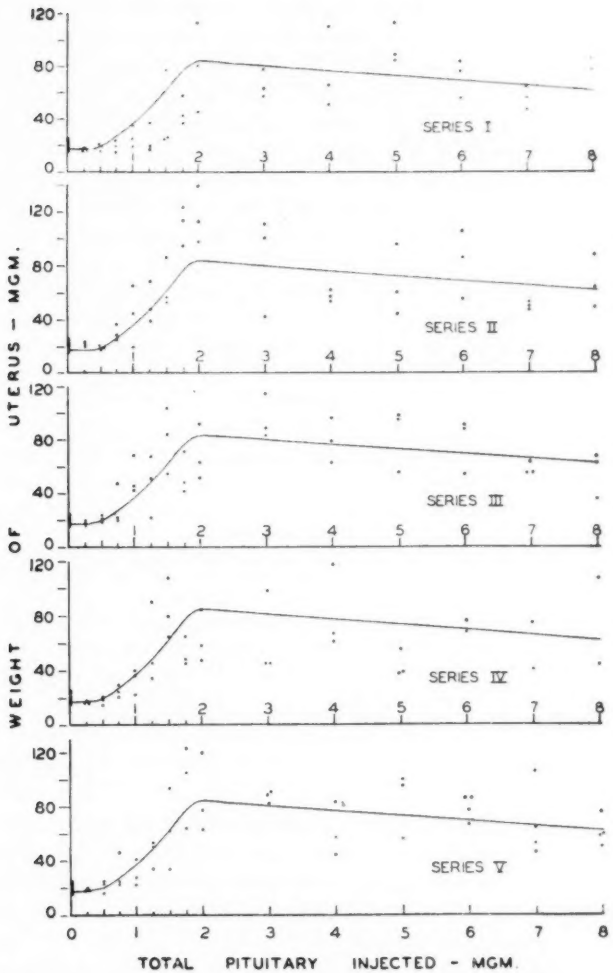

Fig. 6. Uterine weights of each series. The vertical bar on the zero ordinate represents the uninjected control weight range. The curves are a reproduction of figure 5 A, and are shown to facilitate comparison between series.

of injected material which produces definite gross enlargement of the uterus in at least one of three test rats. As is shown in table 5, the first enlargement in series II, III, IV, and V occurred at 0.75 mgm., while for series I the unit dose was found to be 1.0 mgm.

Uterine weight. The unit is defined as the smallest amount which

produces uterine weight above the control maximum (26.8 mgm.) in at least one of three animals, or which gives an average weight increase 50 per cent above control average. On the basis of either definition, the unit dose for series II to V is 0.75 mgm., and for series I, 1.00 mgm. (See table 5.) In figure 6 all uterine weights of each series are plotted. Superimposed on each graph is the curve of the mean uterine weights for all series taken as a whole (see fig. 5a). Table 6 presents the average uterine weights for each series compared with the total averages. With the exception of series I, there is fairly close agreement among the series, especially below the 1.75 mgm. level. Series I shows a slight lag behind the others, suggesting that this batch of pituitaries was somewhat less potent than the other four. The variation on the 0.75 mgm. and 1.0 mgm. doses *within* any given series is not great. Beyond this level, the

TABLE 6

Average uterine weights (without fluid) of each series compared to total average of the combined series

All weights in milligrams

SERIES	DOSE IN MILLIGRAMS														
	Control	0.25	0.50	0.75	1.00	1.25	1.50	1.75	2.0	3.0	4.0	5.0	6.0	7.0	8.0
I	21.6	16.6	18.4	19.6	27.0	23.9	43.2	46.4	82.0	68.7	75.9	95.8	72.7	56.5	76.6
II	18.3	21.8	20.4	29.7	42.2	52.5	65.7	111.2	117.8	84.9	58.3	67.6	83.8	50.5	67.7
III	19.7	17.9	22.3	30.3	52.5	47.5	80.5	53.9	68.4	96.5	79.9	83.6	77.9	58.6	55.9
IV	16.3	17.7	18.5	25.2	33.4	57.5	85.1	53.5	63.6	63.6	82.3	44.3	73.0	58.9	77.7
V	16.8	18.9	21.3	31.7	30.5	46.4	67.2	98.8	86.6	87.3	62.6	85.5	80.3	68.6	62.2
Total..	18.9	18.6	20.2	27.3	37.1	45.6	68.4	72 8	83.6	80.0	71.8	75.2	78.0	59.2	67.0

variation increases considerably. Although each series approximates the curve of the combined series reasonably well, it is only at the very minimal doses (0.75 mgm. and 1.00 mgm.) that close agreement exists. Hence, it is at the minimal levels that the uterine weight curve is most valuable, insofar as our experience is concerned.

Ovarian weight. The unit of minimal ovarian weight increase is defined as the smallest quantity which produces increase in the weight of both ovaries above the control maximum (17.7 mgm.) in at least one of three test animals. This unit is somewhat smaller than the one generally adopted, in which 50 to 100 per cent increase is required. The unit doses (see table 5) are as follows: series I, 7.0 mgm.; series II, 6.0 mgm.; series III, 6.0 mgm.; series IV, 8.0 mgm.; and series V, 5.0 mgm. These figures are in relatively close agreement, again reflecting the uniformity of the potency of the pituitaries from mature female rats, castrated for 20 days.

DISCUSSION. The uterus of the immature rat fulfills our objectives better than the other end-points investigated. It is sensitive to small amounts of gonadotropic substances, permitting the use of relatively large numbers of test animals, and it is reliable. The minimal uterine response, as judged by gross enlargement or weight increase, occurred at the 0.75 mgm. level, while ovarian weight did not increase until the 6.0 mgm. dose was reached. The uterine end-points, in this investigation at least, are therefore about 8 times as sensitive as the ovarian weight end-point.

The data from each series show that the uterine units have been determined with an error of less than ± 0.25 mgm., or less than 33 per cent. The fact that the results of the five series were in such close agreement as regards the unit dose, shows that it is possible to determine the gonadotropic potency accurately with as few as three test animals on each dose level. Using this number of rats per dose, the accuracy of the assay will depend almost entirely upon how closely the doses are graded. Since only the first part of the uterine weight curve is useful, it is suggested that a preliminary assay be run using a single animal at each of rather widely separated dose levels in order to locate approximately the minimal range of response. This done, a final assay can be conducted, concentrating on this narrowed range and employing three or more animals on each of very closely graded doses. In this way the unit dose can be located rather exactly. Either the gross enlargement or weight end-points can be used, but the latter, of course, has the added advantage of being more objective and more readily analyzed statistically.

Where the quantity of material to be assayed is too limited to permit a preliminary assay to be made (as in the case of individual pituitaries or small samples of blood), a combination of uterine and ovarian weight end-points would be useful, provided the ratio between the two units has been determined for the type of material being assayed. For example, in assaying a pituitary from a treated castrate rat, a single test animal responds to a given dose with a 65 mgm. uterus. Reference to the frequency distribution in table 3 shows that this response indicates a potency equal to anywhere from 1 mgm. to 8 mgm. of the 20 day castrate standard pituitary. If, however, the ovaries of this test rat weighed 20 mgm., one would conclude that the potency of that dose was equivalent to about 8 mgm. of the standard pituitary. On the other hand, if a dose three times as large as the one which produced the 65 mgm. uterus was required to elicit a 20 mgm. ovary response in a second test rat, the potency of the original dose was equivalent to about 2 or 3 mgm. of the standard.

Preliminary work in this laboratory suggests that the uterine response to injections of normal female rat pituitary, human menopause blood, and several commercial gonadotropic extracts always occurs at lower doses than the ovarian weight response, but that the ratio between the uterine

and ovarian units, and the shape of the curves obtained, differ depending upon the source of the material. These observations bring to mind the excellent work of Hamburger (13) and of Nelson and Overholser (14), who found differences in the rat-mouse ratio (ovary weight units used) in various gonadotropic preparations. These differences may possibly be related to uterine-ovary weight ratios, and may reflect definite differences in the relative proportions of FSH and LH. Work is now in progress in this laboratory to elucidate some of the factors responsible for these variations in the ratio and shape of the curves.

The mechanism of uterine enlargement obviously involves the ovary. The ovary responds to small doses with increased secretory function without growth, and to larger doses it responds both by increased secretion and growth. That this is true is shown by the uterine enlargement resulting from a dose $\frac{1}{8}$ as large as that required to cause increase in ovary weight. Hence, the uterine end-point measures the capacity of a given gonadotropic substance to stimulate the secretory function of the ovary, while the ovary weight end-point measures its ability to cause ovarian growth. That the pituitary suspensions used in this experiment acted on the uterus through the ovary and not directly was confirmed by administering large doses to ovariectomized test rats. No uterine enlargement resulted. It is interesting to note that with the first uterine enlargement (at the 0.75 mgm. and 1.0 mgm. doses) the mean ovary weights are slightly but significantly less than the control average. (See table 1.) Although it is too early to come to any definite conclusion regarding this decrease, it seems not unlikely that as a result of gonadotropic stimulation the first increase in ovarian secretion is associated with inhibition of ovary growth.

SUMMARY

Two hundred and twenty-eight immature female rats were employed in the assay of aqueous suspensions made from pooled pituitary glands of 57 mature female rats, castrated 20 days previously. Fifteen animals on each of 14 closely-graded dose levels were injected twice daily for $4\frac{1}{2}$ days. The degree of uterine enlargement above control size was judged by gross inspection. Vaginal, uterine, and ovarian weights were recorded, and vaginal opening observed.

The curves of uterine weight (minus fluid) and vaginal weight reveal a close correlation between them. The curves rise rapidly to a maximum and very slowly recede, so that only a small portion, at the lowest dose levels, is useful for assay purposes.

The minimal doses producing uterine enlargement and uterine and vaginal weight increase were about $\frac{1}{3}$ to $\frac{1}{2}$ as large as the minimal dose causing vaginal opening, and only $\frac{1}{8}$ as large as the minimal dose which resulted in ovary weight increase. Vaginal opening was the least de-

pendable, while uterine weight was considered the most satisfactory gonadotropic end-point.

Attention is called to the ratio between the uterine and ovarian units, and to the shape of the uterine weight curve, since there is some evidence to show that these vary among the different gonadotropic preparations, depending upon the source.

ADDENDUM. Since this paper was written, Levin and Tyndale have published their work in complete form (Endocrinology **21**: 619, 1937). Immature mice were injected once daily for three days with extracts of post-menopausal and castrate urine, and the weights of the uteri were noted. Recalculation of their data, for purposes of comparison, in terms of the unit definitions described above, indicates that the ratio of the minimal uterine unit to minimal ovarian unit is approximately 3 or 4 to 1, as contrasted to our ratio of 8 to 1. A further difference is seen in the fact that their maximum uterine weight occurs on a dose which produced a simultaneous ovarian weight increase of 55 per cent, while in our series, the maximum uterine response was produced by doses considerably below that producing ovary weight increase. These differences are probably due to the difference in gonadotropic material and test animals used, and to the fact that their material was subjected to chemical extraction. The difference in number of injections per day is probably also a factor involved.

A footnote to their paper states that "division of the total dose into more injections given at more frequent intervals would probably be better in a physiological sense. However, we feel that the additional labor involved is not justified by the slightly better responses which might result."

We tested this assertion on fifty immature rats, using the same material and procedures as described above, with the exception that the injection period was reduced to three days and with autopsy 72 hours after the first injection, thus duplicating the method of Levin and Tyndale. Half of the group received one injection of 1 cc. per day, while the other half was given two injections of 0.5 cc. each per day. Several dose levels were employed. Uterine response in the latter group occurred at lower doses, was more uniform, and more pronounced, as is illustrated by the two groups receiving 3 mgm. of pituitary suspension. The five rats receiving one injection per day showed uterine weights as follows: 26, 30, 60, 20 and 26 mgm. with an average of 32 mgm., while the uterine weights from the ten rats which received the same total doses from the *same* bottle, but which were injected twice a day, were as follows: 93, 101, 70, 96, 71, 119, 92, 114, 97 and 104 mgm. with average of 89 mgm. Response to smaller doses likewise showed the marked superiority of giving two injections per day. We feel, therefore, that it is highly worth while to inject gonado-

tropic solutions more than once a day, since the resulting improvement is far from being slight.

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CHANGES IN THE WATER OF TISSUES INDUCED BY DIETS CONTAINING VARIOUS MINERAL SUPPLEMENTS[1]

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In the animal organism, water does not move alone but in the company of electrolytes and other solutes. The nature of the dissolved osmotically active substances varies widely among different classes of animals and in different environments. Indeed, the evolution of animal organisms has depended to no small extent upon the ability to maintain osmotic equilibrium (Baldwin, 1937). This concentration of electrolytes and organic compounds dissolved in the body fluids is maintained by a variety of intricate mechanisms not all of which are yet understood.

The knowledge regarding the influence of individual ions on the hydration of tissues is derived mainly from two sources: studies of the hydration of isolated cells and observations of the influence of certain ions on edema. Lucke and McCutcheon (1932) noted that the chlorides of calcium and magnesium decreased the rate of swelling of sea urchin eggs in hypotonic dextrose solution containing electrolytes while the chlorides of sodium, potassium, lithium and ammonium caused the eggs to swell. Similar observations had earlier been made by Osterhout (1922) and J. Loeb (1922–23). In 1923, L. Loeb in summarizing the evidence from experiments of various types, showed that sodium promotes retention of water while potassium and calcium lead to a discharge of edema. The manifold directions in which water plays a rôle in normal body function have been pointed out by Peters (1935).

In a recent study (Eppright and Smith, 1937), it was shown that pronounced and characteristic changes in the composition of the ash of muscle can be brought about by the suitable addition of certain salts or combinations of salts to a basal salt-poor ration. The present report describes the associated changes in the water of the tissues and, from calculation of the

[1] The data forming the basis of this paper were taken from a dissertation submitted by Ercel S. Eppright in partial fulfillment for the degree of Doctor of Philosophy, Yale University, 1936.

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extracellular water, suggests an explanation of the observed changes in tissue electrolytes under the experimental conditions employed.

EXPERIMENTAL. *Animals and diets.* The animals (rats) used for this study, the method of feeding, and the composition of the experimental rations have been previously described (Eppright and Smith, 1937). The general procedure involved the replacement in a low salt diet of some of the salts and groups of salts which are contained in the Osborne-Mendel (1919) salt mixture, the quantities used being determined by the amounts consumed by normal male rats eating *ad libitum* an adequate synthetic ration containing 4 per cent of the salt mixture. The tissues analyzed were taken from rats in the following groups:

Group no.	*Mineral supplements*
I	Osborne-Mendel salt mixture
II	Same minus NaCl and K
III	Ca + P
IV	NaCl + K
V	NaCl
VI	Low salt
VII	$CaCO_3$[2]

Analytical. The tissues of the animals in the various replacement groups were dissected as previously described (Eppright and Smith, 1937), placed in covered tared bottles, weighed, and dried to constant weight in a vacuum oven at 60 to 65°, at a pressure of carbon dioxide corresponding to 20 millimeters of mercury. In the case of larger amounts of tissue, as skin, liver, and muscle, weight was considered constant if on successive weighings, the difference was not greater than 0.1 per cent. Lung, heart, and spleen were considered dry when successive weighings differed no more than 1.0 mgm. The loss of weight was calculated as moisture.

Table 1 shows the average percentage of moisture for each organ for each group. The results were treated statistically, and table 2 permits an evaluation according to the significant ratio.[3]

These data together with those presented by Smith and Smith (1934)

[2] 25.0 mgm. daily.

[3] This is the difference in the means divided by the probable error of the difference

$$m_1 \frac{- m_2}{p.e.d.}$$

m_1 = the mean of one series of values
m_2 = the mean of a second series of values
$p.e.d.$ = $\sqrt{p.e.m_1 + p.e.m_2}$, where $p.e.d.$ is the probable error of the difference and $p.e.m_1$ and $p.e.m_2$ refer to the probable error of the two means respectively.

If the significance ratio is larger than 3.0, the chances are greater than 20 to 1 that the difference of the two means considered is real (Sherman, 1932).

for blood permit the calculation of extracellular water according to the method of Harrison, Darrow and Yannet (1936). Table 3 shows the per cent of extracellular water thus calculated in muscle tissue.

TABLE 1

Summary of water content of fresh tissue expressed in per cent

ORGAN	GROUP I	GROUP II	GROUP III	GROUP IV	GROUP V	GROUP VI	GROUP VII
	Osborne-Mendel salt mixture	Osborne-Mendel salt mixture minus NaCl and K	Ca + P	NaCl + K	NaCl	Low salt	$CaCO_3$
	per cent	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
Adrenal	60.82	61.06	63.01	63.56	65.30	61.16	61.76
Skin	53.41	51.45	52.36	55.07	56.67	53.79	51.33
Kidney	74.81	74.86	76.09	77.93	78.60	76.64	76.54
Lung	77.30	77.19	77.57	78.11	78.80	78.41	77.68
Liver	70.01	69.56	69.65	70.73	71.01	70.03	70.16
Muscle	75.47	74.90	75.07	75.83	75.51	75.54	75.56
Heart	76.97	76.57	76.07	77.53	77.56	77.05	76.84
Spleen	76.71	76.74	76.19	76.93	77.15	77.11	77.13
Number of rats	12	10	12	12	8	13	9

TABLE 2

Significance ratios between water content of tissues: Other groups referred to group I

	ADRENAL	SKIN	KIDNEY	LUNG	LIVER	MUSCLE	HEART	SPLEEN
II (Osborne-Mendel salt mixture minus NaCl and K)	+0.3	−2.2	+0.1	−0.28	−3.4	−3.1	−3.7	+0.2
III (Ca + P)	+5.6	−1.8	+4.9	−0.81	−3.0	−3.3	−5.2	−4.3
IV (NaCl + K)	+3.1	+2.1	+4.6	+2.4	+4.8	+1.7	+4.6	+1.5
V (NaCl)	+6.0	+4.7	+3.5	+4.5	+3.4	+0.2	+4.2	+3.3
VI (Low salt)	+0.8	−0.5	+6.5	+3.5	+0.1	+0.4	+0.6	+3.0
VII ($CaCO_3$)	+1.6	−2.6	+6.4	+1.1	+0.7	+0.8	−0.8	+3.2

Significance ratios between water content of tissues: Group II (Osborne-Mendel salt mixture minus NaCl and K) Referred to group IV (NaCl + K)

	−2.4	−3.9	−4.5	−3.2	−7.8	−4.0	−9.1	−1.3

Significance ratios between water content of tissues: Group III (Ca + P) referred to group V (NaCl)

	−3.0	−7.8	−2.3	−3.4	−4.8	−3.1	−8.2	−8.7

DISCUSSION OF RESULTS. *Group I (Osborne-Mendel salt mixture).* When the complete salt mixture was given, the heart, spleen, and lung con-

tained more water than the other tissues examined, all three having approximately 77 per cent water and maintaining in the individual rats of the group a remarkably constant degree of hydration. Next in order of water content were muscle and kidney; the former was uniform in hydration while the latter was variable. The adrenal glands were conspicuously poor in water and were more variable than other organs and tissues except possibly the skin and kidney. A few moisture determinations made on other endocrine glands indicate that the adrenal glands are unique in these respects; the thymus glands examined contained on the average 77 per cent water, the thyroids 70 to 72 per cent, and the pituitaries 76 to 79 per cent.

Group VI (low salt). Upon withdrawal of salts from the diet, there was a general tendency toward hydration of tissues, although in every

TABLE 3

Extracellular water in muscle—per cent of fresh tissue

GROUP I			GROUP II			GROUP IV			GROUP V			GROUP VI		
Osborne-Mendel salt mixture			Osborne-Mendel salt mixture minus NaCl and K			NaCl + K			NaCl			Low salt		
Number of rats	A*	B†	Number of rats	A	B	Number of rats	A	B	Number of rats	A	B	Number of rats	A	B
2	13.3	13.1‡	2	12.5	11.3	2	11.6	15.1	2	12.7	18.1	2	10.2	13.1
2	12.8	13.6	2	11.2	12.9	2	13.5	17.6	2	13.5	17.5	2	10.1	16.1
2	13.4	12.7	2	12.0	12.8	2	14.6	15.2	2	13.1	18.3	2	10.2	17.5
1	13.2	12.7	1	12.2		1	12.4		1	12.0		1	10.6	18.2
1	13.7		1	11.1					1	13.5		1	10.9	16.2
Average	13.2	13.0		11.8	12.3		13.0	15.9		12.9	17.9		10.4	16.2

* As calculated from chlorides.

† As calculated from sodium.

‡ Only one animal represented.

case the hydration was not so pronounced as found by Swanson and Smith (1936) who used an experimental period of 90, instead of 60 days, as in the present study. The kidney, lung, and spleen were significantly more hydrated than the corresponding organs of group I (Osborne-Mendel salt mixture). The skin, adrenal glands, and heart contained more water in the low salt series but the difference between this and group I was not statistically significant. The water content of muscle and liver was not appreciably different from that of group I.

Group V (NaCl). Upon the addition of sodium chloride to the low salt diet, the hydration of all the tissues except muscle increased greatly in comparison with those of animals given the same amount of sodium chloride in the presence of other salts, as in group I (Osborne-Mendel salt mixture); the differences were statistically valid in every case. The most

significant variations in hydration occurred in the adrenal glands. Next in order were the skin, lung and heart. This retention of water by all tissues except skeletal muscle, following the ingestion of normal amounts of sodium chloride in the absence of other salts, was conspicuous.

Group IV (*NaCl + K*). Potassium exerted a modifying influence upon the water retention induced by sodium chloride, diminishing greatly the hydration of the adrenal glands and reducing that of the skin and lungs to insignificance. It was without effect upon the heart but increased somewhat the water of the skeletal muscles. As with groups V (NaCl) and VI (low salt) the general direction of change was toward excessive hydration.

Group II (*Osborne-Mendel salt mixture minus NaCl and K*). Inspection of tables 1 and 2 shows that the trend of this group as compared with group I (Osborne-Mendel salt mixture) was toward dehydration; the water contents of heart, muscle, and liver were significantly diminished.

Group III (*Ca + P*). The water content of the tissues of group III followed the general pattern of group II in which the mineral supplement was essentially the same except that it was enriched with magnesium and the trace elements. The observation that calcium and its related elements promote dehydration when unaccompanied by sodium and potassium is thereby confirmed. The liver, muscle, heart and spleen were significantly less hydrated than in group I (Osborne-Mendel salt mixture). In fact all of the organs except the kidneys, lungs, and adrenal glands were so affected.

The very significant differences in tissue hydration occurring between the two calcium supplemented groups (II and III) and the sodium chloride and potassium supplemented groups (IV and V) are shown in table 2. The tissues of group II (Osborne-Mendel salt mixture minus NaCl and K) were significantly less hydrated for the most part than the tissues of group IV (NaCl + K); and the tissues of group III (Ca + P), except the kidney, were less hydrated than the tissues of the sodium chloride supplemented group V.

Group VII ($CaCO_3$). From the distribution of water as described in groups II and III, and groups IV and V, it is apparent that calcium and its related ions are antagonistic to sodium chloride and potassium as regards retention of water in tissues. Inasmuch as the basal low salt diet is, relative to calcium, high in sodium and potassium, it seemed possible that a limited amount of calcium (10 mgm.) added to the low salt diet might efface the peculiarities in hydration noted in the low salt group. Among all the groups, this group did show the fewest significant deviations from group I. With the exception of the skin and adrenal glands, this restricted amount of calcium (10 mgm.) resulted in tissues of greater hydration than occurred with more liberal amounts of calcium in the diet as, for instance, in group III with 50 mgm. calcium daily and group II with

99 mgm. daily. Although the addition of this limited amount of calcium did not result in tissues of normal hydration as judged by group I, the conception that the normal hydration of tissues is a function of a balance of electrolytes, particularly calcium, sodium, and potassium, receives some support, inasmuch as the fewest significant differences occurred in this group.

Interrelationships. A gross examination of the data reveals some relationships which, although in many cases not marked, are suggestive. The proportion of water in spleen and heart varied together in four of six groups, when averages were compared with the average hydration of these tissues in group I (Osborne-Mendel salt mixture). Potassium added to the sodium chloride supplement diminished the hydration of spleen but not of the heart. Muscle and liver usually varied together, but with respect to sodium and potassium their relations were reversed; sodium stimulated hydration of liver but not of muscles while potassium stimulated hydration of muscle and not of liver. This behavior is in accordance with the fact that potassium is intimately related to the muscle and the suggestion that the liver is concerned in the metabolism of sodium (Klinke, 1931).

Inspection of the data showed a possible relationship between the hydration of the adrenal glands and that of the organs of excretion, namely, the kidney, skin, and lungs, particularly when added sodium was not balanced by potassium. The coefficient of correlation between the hydration of the adrenal glands of all groups and of the kidneys of all groups was +0.58 which may be regarded as substantial and marked though not high. This observation is of interest in connection with the suggestion that the adrenal glands are concerned in water metabolism through a mechanism which involves control over the kidneys (Harrop, Soffer, Nicholson, and Strauss, 1935).

Extracellular water in muscle. Harrison et al. (1936) have suggested a method for the calculation of extracellular water which depends on the total electrolytes in the tissues, their concentration in serum, and the assumption that the concentration of chloride and, probably, sodium in the extracellular fluids is equivalent to their concentration in serum. In the present study the extracellular water was thus calculated, using the following values for serum electrolytes[4] reported by Smith and Smith (1934):

	Cl	Na
Low salt animals	102.8 ±1.16	130.5 ±0.7
Calorie controls	102.8 ±0.63	131.7 ±0.45

The dietary management of group VI (low salt) and group I (Osborne-Mendel salt mixture) in the present study was practically the same as that of the corresponding animals used in their study of serum. In view of the well-known constancy of the blood, the application of these figures to the rats of this study seems justified. Whether or not they are equally

[4] Expressed in milli-equivalents per liter.

applicable to groups II, IV and V is less certain; the calculated results are presented as suggestive rather than conclusive.

When the extracellular water is calculated on the basis of chlorides the uniformity in the relation of extracellular water to the fresh weight of the muscle is striking throughout the groups. Within a group the variations were seldom greater than 1 or 2 per cent. That the chlorides in the diet may have some influence on the amount of extracellular water is shown by the fact that the values in group VI (low salt) and group II (Osborne-Mendel salt mixture minus NaCl and K) were consistently less than in group I whereas in groups IV (NaCl + K) and V (NaCl), both of which were given chloride supplements, the values were more nearly like those of group I. In other words, the chloride-supplemented groups had more extracellular water in muscle than the groups not given chloride adjuvants, when the calculations were based on the chloride ion.

In group I (Osborne-Mendel salt mixture) the extracellular water as calculated by sodium, agreed very closely with the water estimated on the basis of chloride; i.e., most of the sodium present in the muscles of this group could be accounted for by the sodium in extracellular water as determined from chlorides. In the low salt group (VI) this agreement was not sustained. It is quite apparent that under these conditions of diet there is "excess sodium" which cannot be accounted for in the extracellular fluid as an accompaniment of chlorides. Of further interest is the fact that the addition of calcium and related elements to the diet (as in group II) eliminates this "excess of sodium" found in the low salt group whereas with sodium chloride or sodium chloride plus potassium (as in groups V and IV), the discrepancy remains.

An "excess of sodium" in muscle above that which can be accounted for in extracellular water was described by Harrison et al. (1936), who found a similar excess in dog muscle but in no other tissue except the skeleton. The present study suggests that this excess in muscle is related to the nutritional state; with an adequate intake of inorganic salts, particularly calcium, there is little, if any, excess sodium. In the muscle tissue of monkeys and rabbits, Harrison and co-workers (1936) failed to find the "excess sodium" and concluded that it may be characteristic of the dog. In this connection a study of the state of nutrition of the animals might have been of interest.

The nature of the "excess sodium" presents some questions. The uniformity of extracellular water in all the groups as calculated by chlorides suggests that if the excess sodium is present in the extracellular fluid it exists in an osmotically less effective, perhaps non-ionized form resembling the skeletal sodium described by Harrison et al. (1936). On the other hand, the loss of potassium from the muscle of the groups consuming insufficient calcium and, as herein shown, possessing "excess sodium," was consistently large (Eppright and Smith, 1937) and bespeaks a replacement of the potassium with sodium, in which case the sodium would likely

be intracellular. Since calcium is known to influence permeability of cells, it seems not improbable that under certain nutritional conditions involving extreme lack of calcium, the permeability of the cell is altered. The present observation that a deficiency of calcium appears to bring about the entrance of sodium into the muscles cells (as judged by calculations of extracellular water on the basis of sodium) is in harmony with the previous finding (Eppright and Smith, 1937) that replacement of calcium in the diet of the low salt rate is effective in bringing about a normal K/Na ratio in the muscle.

SUMMARY

The electrolyte balance of the diet influences the hydration of the tissues.

When sodium chloride constitutes the only mineral supplement to the salt-poor basal diet, the tissues, except skeletal muscles, are more hydrated than normal. Potassium exerts a slightly modifying effect.

When the mineral supplement consists mainly of calcium, the general tendency is toward dehydration; liver, muscle, heart, and possibly the spleen, are less hydrated than normal.

The volumes of extracellular water as determined by chloride and by sodium are in close agreement when the diet is adequate in all respects. It appears that, in the absence of calcium and phosphorus, muscle tissue contains more sodium than can be accounted for by that in the extracellular water as calculated from the chloride.

The normal distribution of water in the organism depends to a considerable extent upon the balance of the calcium with its related elements and the alkali metals sodium and potassium. The present study emphasizes the importance of the mineral constituents of the diet in controlling the water of the tissues.

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ROENTGENOLOGIC OBSERVATIONS OF THE SPLEEN OF THE DOG UNDER ETHER, SODIUM AMYTAL, PENTOBARBITAL SODIUM AND PENTOTHAL SODIUM ANESTHESIA

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Markedly large spleens frequently have been observed at laparotomy performed on dogs and on cats anesthetized with sodium amytal or with pentobarbital sodium. It has been noted also that the number of circulating erythrocytes is reduced during anesthesia with these drugs (Bourne, Bruger and Dreyer; Cook and Rose; Adolph and Gerbasi). Searles and Essex have presented evidence that the spleen is chiefly responsible for the reduction in the number of erythrocytes in the peripheral blood of the dog anesthetized with sodium amytal. Essex, Seeley, Higgins and Mann have confirmed this finding. Higgins and Corwin did not find the number of erythrocytes of the rat and the rabbit significantly altered by anesthesia with sodium amytal as compared with results on animals anesthetized with ether.

Changes in the splenic volume could be inferred from the data obtained in previous investigations but to find a more direct method of following changes in the size of the spleen was indicated. Consequently it seemed desirable to determine, by roentgenographic means, alterations in splenic size produced by the anesthetics used routinely in this laboratory. The state of contraction or dilatation of the spleen thus could be correlated with the data on the number of erythrocytes and the amount of hemoglobin in the peripheral circulation.

METHODS. An attempt was made to visualize the spleen roentgenologically following intravenous injections of thorotrast made according to the method of Oka and of Radt. The results were not sufficiently satisfactory to justify further use of this procedure. The work of Steggerda and Gianturco on visualization of various organs by subepithelial injections of thorotrast suggested the possibility of rendering the spleen opaque to roentgen rays by injections of this substance beneath the splenic capsule. As with the technic of Oka and of Radt the shadows were not intense enough for our purpose. Therefore the well known method of Barcroft and Harris was used. The spleen was effectively outlined by small, perforated, disk-shaped pellets of lead which were sewed 2 or 3 cm. apart

along the periphery of the organ under anesthesia and with sterile technic. Three weeks later observations on the effects of the anesthetics were begun.

Food was withheld from the animals for about eighteen hours previous to the experiment. Control roentgenograms were taken after the dog had been allowed to rest quietly on the table for twenty to thirty minutes. With minimal change in the position of the animal the anesthetic was given and successive roentgenographic films were exposed at intervals thereafter. When it was necessary, as in experiments with ether, to remove the animal from the table) care was taken to return the animal as nearly as possible to its original position.

The dose of sodium amytal was 50 mgm. while the dose of pentobarbital sodium was 25 mgm. for each kilogram of body weight. The dose of pentothal sodium (sodium ethyl 1-methyl butyl thiobarbituric acid) was variable but usually 30 mgm. for each kilogram of body weight was enough to produce a short period of surgical anesthesia. Each of the three drugs was given intravenously. Determinations of hemoglobin were made with the Sheard-Sanford hemoglobinometer and standard methods were used in making the erythrocyte counts. The blood was taken from the cephalic, saphenous or jugular vein in the first series of experiments but later it was taken from a puncture in the margin of the ear.

Results. Ether anesthesia causes a marked constriction of the spleen of the dog. This was evident when the control roentgenograms of the quietly resting, unanesthetized animal, were compared with the roentgenograms of the anesthetized animal. The maximal increase in hemoglobin was 2.4 grams per cent and the greatest increase in the number of peripheral erythrocytes was 1,800,000 cells per cubic millimeter of blood. The increased number of erythrocytes and the increased percentage of hemoglobin occurred during the time when the spleen was in a constricted state.

In twelve experiments with sodium amytal the spleen was maximally dilated within thirty minutes following induction of anesthesia (fig. 1a and b). The reduction in the percentage of hemoglobin of the peripheral blood ranged from 1 to 5 grams per cent. The usual decrease was about 2 grams. The maximal decrease in number of peripheral erythrocytes was 2,500,000. Within twenty-four hours, the spleen usually had returned to the size seen under control conditions.

The results of six experiments with pentobarbital sodium were comparable to those with sodium amytal. Maximal dilatation of the spleen usually had occurred twenty to thirty minutes after the anesthetic had been injected. The maximal decrease obtained in the values for hemoglobin was 4 grams per cent. The greatest reduction in the number of peripheral erthrocytes was 2,000,000 below the control value. In some instances six hours and thirty minutes following injection of the anesthetic, the spleen had returned to the size evident in the control roentgenograms.

A small series of experiments was done with pentothal sodium. This drug causes enlargement of the spleen and reduction in the number of erythrocytes and in the concentration of hemoglobin of the peripheral blood comparable to what has been described for sodium amytal and pentobarbital sodium. Since pentothal sodium is a short-acting barbiturate, the effects are of shorter duration. In a typical experiment the spleen was maximally dilated twenty minutes after the anesthetic had been given. The erythrocyte count decreased from a control count of 5,440,000 to 4,755,000 while the concentration of hemoglobin decreased from a control value of 13 grams per cent to 11 grams per cent. In this experiment, two hours after induction of anesthesia the spleen had contracted to the size evident in the control roentgenogram. In one experiment, forty minutes

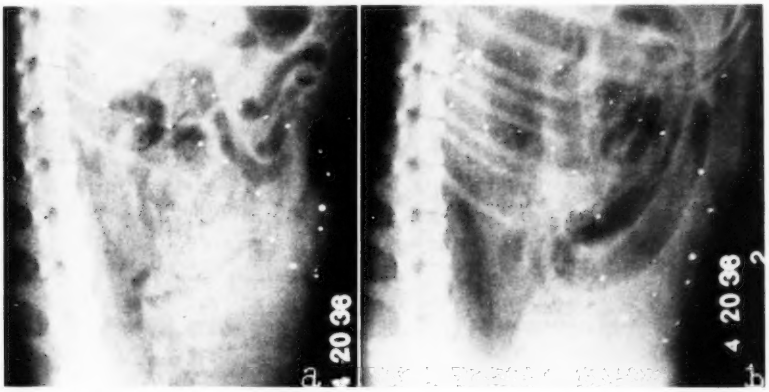

Fig. 1. Roentgenograms of spleen of dog: *a*, taken while animal rested quietly on table; *b*, maximal dilation of spleen during anesthesia with sodium amytal.

after injection of the drug, the spleen was smaller than it had been in the control observation but it dilated again and approximated the size of the spleen under control conditions, one hour after the anesthetic had been given.

In all of the experiments with the barbiturates the marked dilatation of the spleen was associated with a decided reduction in the number of circulating erythrocytes.

Discussion. The spleen is known to decrease greatly in volume in response to a variety of stimuli. Exercise, inhalation of carbon dioxide, anoxemia, ether anesthesia, excitement, stimulation of the splanchnic nerves, drugs such as epinephrine and histamine, and toxins such as rattlesnake and honey bee venoms, cause reduction in splenic volume. An increase in the volume of the spleen is known to follow periods of rest and

quiet. Passive initial enlargement may accompany an increase in blood pressure. The changes in the splenic size under ether anesthesia, and under narcosis by the use of the barbiturates, cannot be satisfactorily explained by the alterations in the blood pressure caused by these anesthetics. The barbiturates cause initially a fall in blood pressure but dilatation of the spleen. The opposite is true in the case of ether. The evidence is conclusive that anesthetics such as amytal and pentobarbital sodium cause dilatation of the spleen and during the process large numbers of erythrocytes are removed from circulation. These anesthetics appear to be specific in the production of this phenomenon. Splenic enlargement does not appear to be mediated through the nervous system since it occurs after denervation of the organ (Searles and Essex). It must be pointed out, however, that complete denervation of the spleen is difficult of accomplishment.

We have no explanation of the mechanism involved in enlargement of the spleen. Anesthesia with the barbiturates, and hibernation, have similar effects on the size of the spleens of certain animals. It is known through the work of Mann and Drips that the spleens of hibernating animals are enlarged beyond what is seen in the nonhibernating animal of the same species. In both conditions respiration is depressed and metabolism is reduced. Some such mechanism as that which operates in the hibernating animal may be responsible for the enlarged spleens seen in certain animals under anesthesia with the barbiturates.

Other organs, such as the liver, may respond in a similar manner to anesthesia with the barbiturates but it is probable that no other organ is affected to such a profound degree as the spleen.

SUMMARY AND CONCLUSIONS

After outlining the spleen with pellets of lead by the method of Barcroft and Harris, a series of observations by means of roentgenograms was made on the size of the spleen of the dog before and during anesthesia with ether, sodium amytal, pentobarbital sodium, and pentothal sodium. Data were obtained at the same time on the percentage of hemoglobin and the number of erythrocytes in the peripheral blood.

Ether produces a profound reduction in the size of the spleen which is accompanied by marked increase in the concentration of hemoglobin and in the number of erythrocytes of the peripheral blood. Anesthesia with sodium amytal, pentobarbital sodium or pentothal sodium causes the opposite effect. Each of these three drugs causes marked enlargement of the spleen and decided reduction in the concentration of hemoglobin and number of erythrocytes of the peripheral blood. During the period of maximal dilatation of the spleen a minimal number of erythrocytes appear in the peripheral blood. The barbiturates seem to be specific in producing enlargement of the spleen.

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THE EFFECTS OF UNILATERAL AND BILATERAL LABYRINTHECTOMY IN MONKEY, BABOON AND CHIMPANZEE[1]

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Section of the eighth nerve in monkeys was first reported by Ferrier and Turner in 1898. They sectioned the nerve distal to the accessory auditory "ganglion" in two monkeys and proximal to this nucleus in two other monkeys. Following the operation they observed rolling movements toward the operated side and a rotation of the head with the chin pointing to the normal side. Beyer and Lewandowsky (1906), Magnus (1922) and, most recently, Northington and Barrera (1934) have described the effects of labyrinthectomy in *Macaca mulatta* (rhesus monkey). The last authors studied unilateral and simultaneous bilateral labyrinthectomy; and have shown that labyrinthectomy and section of the eighth nerve are followed by identical syndromes.

Dandy (1928), Cairns and Brain (1933), Coleman and Lyerly (1933), McKenzie (1936) and others have described the effects of section of the acoustic nerve or its vestibular portion in man. The existence of abnormal vestibular function preoperatively in these cases of Menière's disease must be emphasized.

It is well known that the severity of the disorder resulting from such a lesion diminishes as one ascends the phylogenetic scale. Labyrinthectomy in the baboon and chimpanzee commands attention because the difference between the effects of labyrinthectomy, or acoustic nerve section in man, and in the lower primates, is so striking.

MATERIALS AND METHODS. Ten *Macaca mulatta* (rhesus monkeys), one *Cercopithecus aethiops pygerythrus* (green monkey), one *Papio papio* (baboon), and one *Pan satyrus* (chimpanzee) have been used in this study. Four of the animals had been subjected to previous operations involving extirpation of parts of the cerebellar cortex. In only one of these, an experiment in which the entire anterior lobe of the cerebellum had been removed, was there any detectable difference from those animals in which the labyrinthectomy was a primary procedure. This animal was not included in the following report. In one monkey the eighth nerve was sectioned intracranially.

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The operations were done by an approach through the mastoid process. The facial nerve was cut. The middle ear was exposed and the vestibule opened widely. The nerves entering it were separated from their bony attachment and the posteromedial wall of the vestibule was removed so that the cut ends of the nerves could be seen to be free in the internal auditory meatus. A head lamp and a binocular loupe were necessary during this part of the operation. Although in every case this potentially infected wound was flooded with cerebrospinal fluid, in only two out of more than twenty labyrinthectomies did meningitis occur. In both these animals advanced pulmonary disease was discovered at autopsy. Sodium amytal, nembutal, or ether was used for anesthesia.

Examination of the animals was as complete as possible. This included caloric and rotatory tests for nystagmus in most cases. Many were observed while swimming and while on a horizontal and vertical bar both blindfolded and unblindfolded. Moving pictures were taken in some instances. The sites of the labyrinthectomy were removed and examined histologically in four cases, serial sections being made in one instance. In every case examined histologically the destruction of the vestibule was complete and the site of extirpation was filled with scar tissue.

RESULTS. *Unilateral labyrinthectomy.* In the *Macaca mulatta* unilateral labyrinthectomy was followed by disorders of posture and movement essentially as described by previous investigators. During the recovery from the anesthetic there was a conjugate deviation of the eyes to the side of the operation, and the animal usually lay on that side. As the effect of the anesthetic diminished, a deviation of the head toward the operated side appeared together with a rotation with the occiput to the same side and the chin pointing toward the opposite shoulder. This was soon followed by a continuous horizontal nystagmus, with the quick component toward the normal side, which persisted for about 24 hours, and following this one occasionally saw a few nystagmoid jerks for a variable period, never more than two days. Nystagmus was increased by excitement and by the monkey's moving its eyes toward the normal side. Especially during the later stages of the nystagmus period, the nystagmoid movements were at times rotatory as well as horizontal. In several instances a slight skew deviation of the eyes was observed by one examiner.[3] This was never seen after the first 24 hours postoperatively.

As soon as the animal was able to sit up or to stand, in addition to the abnormal head posture, and probably secondary to it (Magnus, 1924), the extremities on the operated side were flexed and adducted, and on the normal side extended and abducted. First attempts at walking and running were characterized by falling, deviation and circling toward the operated side. Spiralling about the longitudinal axis of the body in jumping, swimming and climbing a vertical bar was consistently present. This was always in the same direction as the head rotation, that is, the back rotated toward the side of the labyrinthectomy. All these symptoms

[3] Dr. Morris Bender who studied the effects of section of the facial nerve in these cases.

were accentuated and detectable longer postoperatively if the examination was made with the animal blindfolded. The ability to walk a narrow horizontal bar usually returned after two weeks. Spiralling on the vertical bar and in the water and deviation toward the side of the lesion persisted longer. The rotation of the head was apparently permanent although the degree of rotation was variable. Repeated rolling movements, as seen in lower animals, did not occur.

As others have pointed out, these animals show less abnormality both of posture and movement when seen clinging to their cages or when climbing about than when standing, walking or running on the floor. They will rarely walk on a flat surface if the cage wall or some other object is available to which they may cling. Although all monkeys have this tendency to some extent, it is more obvious in an animal with a disorder of equilibration. Even when quite wild they will allow themselves to be captured clinging to some object rather than abandon such a secure position by leaping a short distance or running over the floor.

A B

Fig. 1. A. A green monkey (P.V. no. 32), 43 days after destruction of the left labyrinth. Note the rotation of the head with the occiput to the left.

B. The same animal (P.V. no. 32) one day after destruction of the right labyrinth. Note symmetrical position of the head.

Although abnormal postures, as described, are due to differences in muscle tone, these differences were not demonstrable by palpation of the muscles, or by comparing the resistance to passive manipulation of the extremities on the two sides.

Righting reactions were present and occurred in either direction when the animal was on the floor. Soon after the operation there was generally a delay in righting when the operated side was down. Caloric tests on the side of the lesion uniformly failed to produce any nystagmus. Rotation tests were not done following unilateral labyrinthectomy. In all cases an ipsilateral facial paralysis was present.

Although there was considerable variation in the severity of the symp-

toms from animal to animal within the rhesus group, the disability in the baboon and chimpanzee was so much less that it merits special mention. The green monkey (fig. 1A) in some respects seemed more severely affected, but in it the differences were slight. In the baboon (fig. 2A) the eye deviation, the nystagmus and the degree of the abnormal head posture were not essentially different. The other symptoms described were apparently less severe, and disabling, and disappeared more rapidly. The animal was never observed to fall, although it was cautious in its movements for four or five weeks after the operation. On the eighth postoperative day and thereafter it no longer exhibited any tendency to deviate or circle in walking or running and it no longer clung to adjacent objects

Fig. 2 A

Fig. 2 B

Fig. 3

Fig. 2, A. A baboon (P.V. no. 25) 22 days after destruction of the right labyrinth. Note the rotation of the head with the occiput to the right.

B. The same animal (P.V. no. 25) 11 days after destruction of the left labyrinth. Note symmetrical position of the head.

Fig. 3. *Macaca mulatta* (P.V. no. 3) 35 days after the second labyrinthectomy exhibiting delayed righting of the head. If the animal is frightened or attracted by some visual stimulus it would usually place the head in a normal position.

for support. The rhesus monkeys clung to surrounding objects and deviated in running for approximately three weeks postoperatively.

The effects of labyrinthectomy in the chimpanzee were studied in one female who had advanced pulmonary tuberculosis and unfortunately died of meningitis after the second labyrinthectomy. It could not be handled and observations were restricted to what could be seen during its cage activity, and while it ran about a large room and down a long narrow corridor.

The animal lay on the operated side during recovery from the anesthetic and, if disturbed, would return again to the former position. Deviation of the eyes toward the operated side was seen during this period. Nystagmus was transitory and seen only when the animal looked toward the normal side. When present it was horizontal in direction with the quick

component toward the normal side. No nystagmus was observed after the first 24 hours. The rotation of the head was, as in the other animals, with the occiput to the side of the lesion and the chin pointing toward the opposite shoulder. It was slight and was accompanied by a slight flexion of the neck to the operated side. This rotation persisted for about six weeks and intermittently thereafter. The animal was seen to fall three times during the first day after the operation but not in any particular direction and never as a "forced" movement such as occurred in the monkeys. It showed no spiralling or circling when walking. After the second postoperative day no tendency to cling to its cage or to surrounding objects was observed. It showed a tendency, which was well corrected, to deviate toward the side of the lesion when running down a long corridor. This was not present after the third postoperative day. From this time on, aside from the slight abnormal posture of the head, the only abnormality was a tendency to run obliquely with the hind part of the body deviated toward the operated side. Although chimpanzees often run with the posterior extremities tracking to one or the other side of the anterior extremities, they never do so consistently in one direction. Even this slight abnormality had disappeared by the end of the two month period of study.

Postoperative symptoms following section of the acoustic nerve in Menière's disease have been described by Cairns and Brain (1933), Coleman and Lyerly (1933), and most recently, McKenzie (1936). In all the cases described, the effects of such operations were mild and transitory. There was some indication that the severity of postoperative symptoms was greater in those cases in which caloric and auditory tests showed more nearly normal function preoperatively (Cairns and Brain, 1933). In some cases no postoperative symptoms were noted. There was usually horizontal nystagmus with the quick component toward the contralateral side, a fleeting type of diplopia and occasionally skew deviation (Cairns and Brain, 1933). These symptoms were never marked and were usually present for two to five days. During the first two or three days the patient might stagger and deviate toward the side of the operation. This tendency was noted in the dark and with the eyes shut, in one case as long as three months postoperatively. In some cases the patients complained of subjective feelings of unsteadiness although no abnormality was apparent to the examiner (McKenzie, 1936).

Bilateral labyrinthectomy. In seven monkeys, 6 macaques and 1 green monkey, the second labyrinth was destroyed after a variable interval of from 4 hours to 57 days following the first labyrinthectomy. In one monkey both labyrinths were destroyed simultaneously.

Except in the animal in which the interval was but 4 hours, all the monkeys exhibited a horizontal nystagmus after the second labyrinth-

ectomy. This was associated with a conjugate deviation of the eyes to the side of the second operation. The quick component was toward the opposite side and the nystagmus was increased when the monkey moved its eyes in the direction of the quick component. There was usually a temporary rotation and deviation of the head to the side of the second operation. This had, in most cases, disappeared within 6 hours postoperatively although the nystagmus persisted for approximately 24 hours. After 24 hours, except for a tendency to spiral toward the side of the second operation when placed in a tank of water, no consistent asymmetries of posture or movement were seen.

The animals were able to walk soon after recovery from the anesthetic. At first they used a sprawling, abducted gait which gradually improved over a period of several weeks. Progressive movements were poorly coordinated and in some instances there was a tendency to walk backward, particularly when blindfolded. The animals clung tenaciously to the cage wall. All the defects of posture or movement were accentuated if the animals were observed on a flat surface rather than in the cage. Attempts to leap from the cage wall to the floor were seen in only two instances. These resulted in severe falls due to a failure of reflex extension of the legs to the vertical movement downward. The animals were unable to walk upon a narrow horizontal bar either blindfolded or unblindfolded during the 5 week period of observations.

During the first 3 or 4 weeks postoperatively a marked head tremor was noted. This was in every plane, from side to side, in an anterior posterior direction, and especially rotatory. There was a definite hypotonia, as estimated by resistance to passive manipulation and palpation, in the muscles of the neck and shoulder girdle. Occasionally the head was seen in bizarre positions. These, as noted by Northington and Barrera (1934), were more conspicuous when the labyrinthectomies were performed simultaneously.

The importance of vision in the compensation following labyrinthectomy in higher animals has been emphasized by many previous workers. In the monkeys studied here every animal when blindfolded following the second labyrinthectomy, struggled almost constantly to remove the blindfold. This was a behavior not seen regularly in normal monkeys and rarely in animals with as severe disequilibration following a cerebellar operation. Although there was a definite delay in the righting movements of the head when the animal was held by the pelvis in a lateral position (fig. 3) head righting was at times seen when the animal was unblindfolded. When blindfolded head righting under these conditions did not occur. Their ability to stay above water was also influenced by vision. A normal monkey, whether blindfolded or unblindfolded, swam without difficulty with the trunk parallel to the surface of the water and the head well out

of water. A monkey without labyrinths, if unblindfolded could usually get out of a shallow tank and could swim after a fashion so long as it was not submerged. Once under water, or if put in the water blindfolded, it was completely disoriented and would have drowned if not removed.

All vestibular reflexes tested were absent. These included movements of the extremities following vertical movements of the trunk and head. This was studied particularly by allowing the blindfolded animals to slide down an inclined plane in a lateral position. Normal animals when tested thus, showed an extension of the extremities which perfectly balanced the momentum of the body. Animals without labyrinths, lacking such extension when blindfolded, fell away from the inclined plane and at times rolled completely over as an inert mass. In every case post-rotatory nystagmus was absent. During rotation, if the animal was turned slowly, a visual nystagmus was seen, provided the animal looked at some object not on the rotating table. Hot and cold water injected into the external auditory meati failed in every instance to induce any ocular deviation or subsequent nystagmus.

Although in every instance all the vestibular reflexes were lost permanently, there was considerable improvement in the gait and other early symptoms. After about four weeks these reached a steady level where improvement was so slow that it was almost imperceptible between examination periods several days apart. In the experiment where both labyrinths were destroyed at one operation all the symptoms of the disorder, as described above, were more severe and more persistent. In this instance no nystagmus and no consistent asymmetry of posture or movement were detected. However, as stated above, the tendency for the animal to assume bizarre head postures was greater. Recovery in this monkey was definitely slower, requiring approximately two months to reach the same stage of recovery seen in the other animals of the same species 4 weeks after the second operation.

In the baboon the disability resulting from the second labyrinthectomy was much less severe than in the rhesus monkeys. There was a sprawling gait only on the day of the operation, and no hypotonia. Head tremor, which had been present when at rest and with every movement in the macaque, was seen for a few days and then only when the baboon was excited or attempted to turn quickly. The animal was never observed to fall and its movements of progression were better coördinated in every stage of postoperative recovery than in the macaque. All reflexes which depended on purely vestibular function were absent. For example, head righting did not occur when the baboon was blindfolded and held by the pelvis in the lateral position; there was no extension to vertical movements downward when blindfolded; there was no post-rotatory or caloric nystagmus; and the animal was completely unable to swim both blindfolded and

unblindfolded. It would appear then that in this species mechanisms other than vestibular are dominant. Fulton and Keller (1933) have shown that, in a comparative study of the sign of Babinski, the baboon exhibits more cortical dominance than does the macaque.

Observations on the chimpanzee were marred by the onset of meningitis after the second labyrinthectomy. However, our observations in this species confirm those after unilateral labyrinthectomy, i.e., that the neurological disorder resulting from labyrinthine destruction is strikingly less in the anthropoids than in the monkeys. The effects of bilateral labyrinthectomy or eighth nerve section in man have not, in our knowledge, been reported.

CONCLUSIONS

The effects of unilateral and bilateral labyrinthectomy in the rhesus monkey, *Macaca mulatta*, were similar to those described by previous workers. The effects of labyrinthectomy in the chimpanzee more closely resembled those in man than those in the monkeys. The baboon was found to have an intermediate position between the chimpanzee and the macaque in respect to this neurological disorder.

The Author wishes to express his gratitude to Dr. John F. Fulton, in whose laboratory this work was done, for his assistance in the work.

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THE RESPONSE OF SINGLE OPTIC NERVE FIBERS OF THE VERTEBRATE EYE TO ILLUMINATION OF THE RETINA

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In a series of three papers Adrian and Matthews (1927, 1928) presented a study of the discharge of impulses in the optic nerve of the eel's eye, and so opened a new approach to problems of visual physiology. In those papers the simultaneous activity of large numbers of optic nerve fibers was recorded. The possibility of extending that work to an analysis of the activity in single optic nerve fibers was suggested by the subsequent investigation of Hartline and Graham (1932) on the optic nerve fibers of a primitive arthropod eye (*Limulus*). The present paper describes the discharge of impulses in single optic nerve fibers of the cold-blooded vertebrate eye, in response to illumination of the retina.

METHOD. The usual methods for obtaining action potentials from only one fiber in a nerve trunk have not succeeded when applied to the vertebrate optic nerve. It is therefore necessary to utilize the intra-ocular portion of the optic nerve fibers, by exposing the fundus of the eye and dissecting small bundles of fibers from the anterior (vitreous) surface of the retina, where they converge to the head of the optic nerve.

From a freshly pithed animal (in most of these experiments large bull-frogs (*Rana catesbiana*) were used) an eye is excised, pinned fundus down in a moist chamber, and its anterior half (cornea, lens, choroid body) removed. A wide V-shaped cut extending almost to the nerve head gives access to the fundus and permits the vitreous humor to be drained away. It is then possible to dissect free small bundles of nerve fibers for a length of 1 to 2 mm., and these may be further dissected until only one fiber remains active. A bundle is severed where it enters the nerve head and the cut end lifted up onto one of the electrode wicks; the second electrode is diffuse, touching the surface of the retina. The action potentials from such bundles of nerve fibers are amplified and recorded by means of an oscillograph. The preparation is mounted in a light-tight, electrically shielded box, in the hollow walls of which water at constant temperature (*ca.* 20°C.) is circulated.

Prepared in this manner the retinas of most cold blooded vertebrate eyes survive for 4–8 hours, as evidenced both by the normal type of retinal action potentials which can be obtained from them (*cf.* Chaffee, Bovie and Hampson, 1923) and by the normal responses and lack of spontaneous activity in nerve fiber bundles freshly dissected from the retina. Fine bundles themselves, after having been lifted away from the retina, rarely remain conducting for more than an hour or two, and usually fail to show action potentials in a much shorter time. Attempts to obtain single fibers are successful in only a very small percentage of trials.

Light from a concentrated tungsten filament lamp is focussed by a condensing lens 12 mm. in diameter upon an objective lens, which in turn forms an image ($\frac{1}{3}$ size) of the illuminated condensing lens upon the retina. A 45° prism close to the objective lens reflects the light downward onto the retina; it can be rotated so as to project the rays at approximately normal incidence onto that portion of the retina from which the nerve fibers in any particular bundle come. At the condensing lens can be placed a suitable diaphragm to limit the size and shape of the illuminated area; this diaphragm is carried on crossed micrometers controlling its position to within 0.01 mm., and its image is accurately focussed on the retina by micrometer movements carrying the preparation itself. The intensity of illumination upon the retina can be reduced by known amounts with Wratten Neutral Tint filters; the full intensity (no filters in the beam) is $2 \cdot 10^4$ meter candles.

Results. The discharge of impulses in any moderately large bundle of intraocular optic nerve fibers of the vertebrate eye is similar to that previously described for the whole optic nerve. Beginning several hundredths of a second after the light is turned on, there is a strong, brief outburst of impulses. Impulses continue to be discharged at a low rate as long as light continues to shine, and when it is turned off there is a renewed vigorous outburst which subsides gradually. Such responses were initially described by Adrian and Matthews (1927) in the eel's optic nerve and subsequently recorded by Granit (1933) from the mammalian optic nerve. It is not until the bundles have been dissected down until only one, or at most only a few, fibers remain active that a new and striking property of the vertebrate optic response is revealed. For such experiments show conclusively that not all of the optic nerve fibers give the same kind of response to light. This diversity of response among fibers from closely adjacent regions of the same retina is extreme and unmistakable; it does not depend upon local conditions of stimulation or adaptation, but appears to be an inherent property of the individual ganglion cells themselves.

Types of response. In figure 1 are records of the action potentials in single optic nerve fibers of the frog's retina. Figure 1A shows a response

in which the initial burst of impulses, at high frequency, is followed by a steady discharge at much lower frequency which lasts throughout the duration of illumination. When the light is turned off this discharge stops. Such a response is similar to that from a simple photoreceptor, observed in *Limulus* optic nerve fibers, even to the short pause following the initial burst. But while *Limulus* optic nerve fibers invariably show this type of response, in the frog's retina it is obtained in less than 20 per cent of the fibers. The other fibers show strikingly different types of response. At least 50 per cent respond (fig. 1B) with a short burst of impulses at high frequency when the light is turned on, but show no im-

Fig. 1. Oscillographic records of the action potentials in three single intraocular optic nerve fibers of the frog's eye, showing three characteristic response types.

A. Response to illumination of the retina consisting of an initial burst of impulses, followed by a maintained discharge lasting throughout illumination. There is no response to cessation of illumination in this fiber (the off response in this record is partly due to retinal potential, partly to another fiber which discharged several small impulses). See also figure 6.

B. Response only to onset and cessation of light. See also figure 2, figure 5A and C.

C. Response only to cessation of illumination. See also figure 5B, D and E.

In this and subsequent records, the time is marked in $\frac{1}{5}$ second, and the signal marking the period of illumination fills the white line immediately above the time marker.

pulses as long as it continues to shine steadily; when the light is turned off there is another brief outburst of impulses. Such responses constitute the most prominent feature of the vertebrate optic response—bursts of impulses occurring only when the conditions of illumination on the retina are changed abruptly. In a third type of response, occurring in about 30 per cent of the fibers, no impulses appear at all during illumination, but there is a vigorous discharge of impulses when the light is turned off (fig. 1C). This discharge may last many seconds, and usually subsides gradually. It is undoubtedly responsible for most of the "off" response obtained from the whole optic nerve.

Most of the optic nerve fibers in the vertebrate eye give responses similar

to one or another of the above described types. These categories, however, are not absolutely rigid, and it would be a mistake to ignore the occasional fiber whose response is intermediate in character. Thus it is not infrequently found that a fiber whose response is of the first type has a maintained level of discharge of a low and irregular frequency, which tends to adapt out after several seconds. No clear-cut cases have been found in which a fiber giving an initial burst followed by a maintained discharge has shown an "off" response; the doubtful cases which have been recorded could have been due to a second fiber, as is the case in figure 1A (see fig. 6A for a clear-cut case, where there is no additional fiber giving an "off" effect). Fibers have been found, however, whose response lacks the initial burst; in these rare cases the discharge builds up slowly (in 2–3 sec.) to a steady level of *ca.* 20 to 30 per sec. When the light is turned off these fibers do show an "off" effect—a distinct increase in frequency for several tenths of a second before the discharge subsides. It is true that fibers which respond predominantly to a change in intensity with "on" and "off" bursts may occasionally show very irregularly scattered impulses or bursts of two or three impulses every few seconds during steady illumination. This, however, is quite different from the regular discharge of figure 1A, and may be regarded as an atypical, intermediate variety of response. Furthermore, fibers showing predominantly an "off" response are occasionally found in which a few scattered impulses "escape" during prolonged illumination.

From these observations it follows that the responses of the entire optic nerve are complex, containing different contributions from different fibers. An analysis of the optic response must therefore take up the properties of the different types of single fiber response separately.

Effect of intensity. Chief among the factors governing the response in any single fiber is the intensity of illumination with which the retina is stimulated. In figure 2 is shown a series of records of the responses to lights of different intensity of a single fiber of the "on-off" response type. With higher intensities of light the responses show shorter latent periods, higher maximum frequencies of discharge and greater numbers of impulses in both the "on" and the "off" bursts. This holds from threshold to intensities 4–5 logarithmic units above it. In figure 3 are plotted, on the left, the reciprocals of the latent periods and, on the right, the frequencies of the discharges (measured from the first six impulses) for both the "on" and the "off" bursts in this same fiber. In this fiber the threshold intensity was the same for both the "on" and the "off" bursts; frequently, however, one or the other of the bursts may have a somewhat lower threshold (usually within one logarithmic unit). It is to be noted that the curves rise abruptly from threshold—frequently the weakest response obtainable has a fairly large number of impulses, at a fairly high frequency.

While the threshold is sharp, it is not always reliable, and may fluctuate in spite of carefully controlled conditions.

The experiment of figure 2 and figure 3 shows the very considerable range over which intensity is effective in speeding up the discharge. This might be expected in view of the wide range which is covered by single

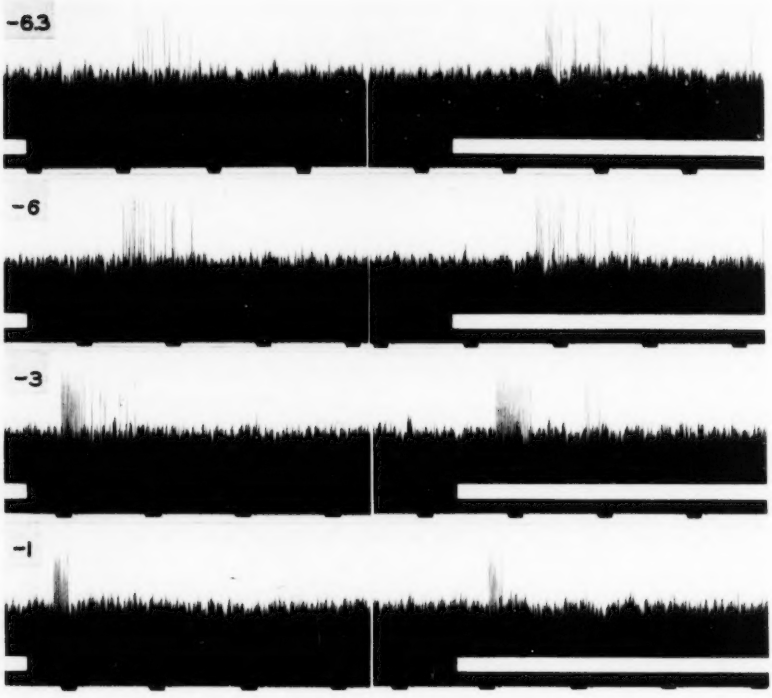

Fig. 2. Effect of intensity of retinal illumination upon the discharge of impulses in a single optic nerve fiber of the frog's eye. Logarithm of the intensity indicated on each record (unit intensity = $2 \cdot 10^4$ meter candles, in this and subsequent figures). Diameter of the spot of light = 0.10 mm. Portions of the records, representing 2 to 3 seconds in each case, have been removed. They contained no impulses, except for the −6 record, which showed four impulses scattered over 2.5 seconds.

visual sense cells in the *Limulus* eye (Hartline and Graham, 1932). This experiment also shows the characteristic effect of very high intensity in reducing the number of impulses and often the frequency of discharge in the bursts. This is especially pronounced for the "off" burst, which may be entirely missing at the highest intensities available.

Similar effects of intensity can be shown for the other types of response.

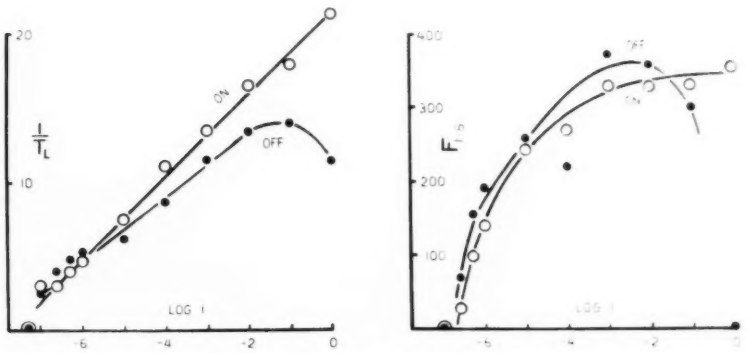

Fig. 3. The relation between intensity of stimulating light and response in a single optic nerve fiber of the frog's eye. Data from experiment of figure 2. On left the ordinates give reciprocal latent period (T_L, secs.); circles give values for "on" burst, dots for "off" burst. On right, ordinates give frequency (impulses per sec.) for the first six impulses of the bursts; circles give values for "on" burst, dots for "off" burst. Abscissae, in both graphs, give the logarithms (base 10) of the stimulating intensity. When no response appears, $1/T_L$ is assigned a value of zero; if fewer than 6 impulses are discharged, F_{1-6} is given a value of zero.

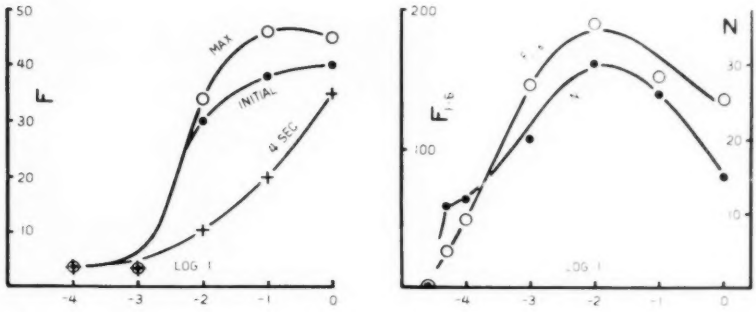

Fig. 4 A. The relation between the logarithm of intensity of retinal illumination (abscissae) and frequency of response (ordinates) in a single optic nerve fiber of the frog's eye. This fiber gave a response consisting of an initial burst followed by a discharge which was maintained throughout illumination. Dots and circles give, respectively, initial and maximum frequencies (three impulses) of initial burst; crosses give the frequency attained after four seconds of steady illumination.

Fig. 4 B. The relation between intensity and response of a single optic nerve fiber in the frog's eye. This fiber gave a response only upon cessation of illumination. Abscissae give the logarithms of the intensity of light. Ordinates (F_{1-6}, on the left) for the circles give the frequency (impulses per second) for the first six impulses; ordinates (N, on the right) for the dots give the total number of impulses in the burst.

In figure 4A are plotted the frequencies of various parts of the discharge in a fiber giving an initial burst followed by a maintained discharge. The initial frequency (first three impulses) and maximum frequency (usually between the 5th to 8th impulse) of the initial burst are given, together with the final level of frequency attained after four seconds of steady illumination. This graph may be compared with a similar plot for the *Limulus* single optic nerve fiber (Hartline and Graham, 1932, fig. 6). At low intensities, near threshold, the maintained discharge usually adapts out completely after a second or two, and often only the initial burst can be obtained.

The degree of activity in a fiber giving only an "off" response is also dependent upon the intensity of the light. In figure 4B are plotted the initial frequencies of the discharge from such a fiber, in response to cessation of lights of different intensities. The sharp threshold, and the diminshed values at high intensities are similar to the corresponding plot of the "off" burst of figure 3. This particular fiber gave a rather short, sharply defined burst not unlike the "off" response in a typical "on-off" fiber. This enabled a reliable count to be made of the total numbers of impulses in the bursts, also plotted in figure 4B. Usually, however, as has been said, the discharge in a fiber giving only an "off" response is prolonged, and dies down very gradually. (Compare fig. 1B with C; fig. 5A and C with B, D and E.) The higher the intensity the longer does the discharge last; at very high intensities the initial part of it may be reduced to a few impulses, but after several seconds it is gradually resumed, and may persist for many minutes at a frequency which may be as high as twenty or thirty impulses per second. Several instances have been observed where such a discharge broke up into rhythmic bursts, coming at about 3 to 5 per second.

Change in intensity. The amount of change in intensity also affects the magnitude of the responses. Fibers which discharge a brief burst of impulses in response to the onset and cessation of illumination also respond to a sudden increase or decrease in its intensity (fig. 5A); the bursts, however, have fewer impulses at a lower frequency. Likewise the "off" fibers will respond, though less strongly, if the illumination is partially reduced (fig. 5B) but not if it is increased in intensity. The frequency of the maintained discharge in those fibers which show such a response is of course determined only by the level of the steady illumination—an increase or decrease in intensity results in a corresponding rise or fall in frequency (fig. 7C).

Light and dark adaptation. A given intensity of light is effectively weaker in the light adapted retina than after dark adaptation. As dark adaptation proceeds the response to a given intensity increases, and correspondingly the threshold falls, rapidly at first and then more gradually.

After one half hour in the dark a fiber may respond to light 1/100 to 1/1000 the intensity of that necessary to elicit a response in the light adapted condition. The essential character of the response in any given fiber, however, is unchanged by adaptation. Reliable quantitative meas-

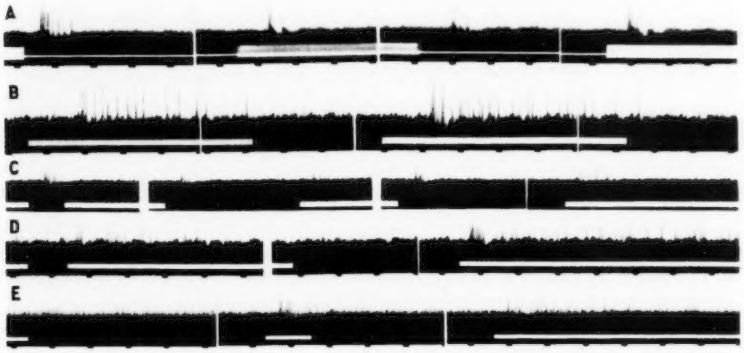

Fig. 5. Records of the impulse discharge in single optic nerve fibers of vertebrate eyes. Record B is from an alligator, the rest are from frogs. Recording as in figure 1.

A. Response to a partial reduction and to an increase in intensity of an "on-off" fiber. Left to right: Onset of light (log I = −2.0); intensity reduced by 0.5 log unit; intensity increased to original value; light turned off.

B. Response to partial reduction in intensity in a fiber giving only "off" responses. Left to right: light of an intensity log I = −2.0 reduced in intensity by 0.7 log unit; (a length of record equivalent to ½ sec., showing a gradually decreasing discharge, has been removed) light increased to original intensity; (a length of record equivalent to 1 sec., showing no impulses, has been removed) light turned off; (a length of record equivalent to 1 sec., showing a steadily decreasing discharge, has been removed) light turned on again.

C. (Time scale same as in the other records.) Effect of exposure in producing an "off" burst in an "on-off" fiber. Left to right: exposures, to a constant intensity, of 0.18 sec., 0.70 sec., 3.0 sec. Exposures made successively within about 10 seconds of each other, hence the "on" bursts in last two records are somewhat weaker than in first record, taken in the completely dark adapted condition.

D. Same as C for a fiber giving only an "off" response. Left to right: exposure to a constant intensity of 0.20 sec. and of 2.2 sec.

E. Inhibition of "off" discharge by re-illumination. (See also B of this fig.). After an interruption of 0.23 sec. light is turned on again (middle section of record); full "off" response shown in last section of record.

urements of the effect of adaptation in these experiments are difficult to obtain, owing to the comparatively short time fibers stay alive in a dissected bundle.

Duration of exposure. The strength of an "off" response at a given intensity depends on the length of time the preceding light has been al-

lowed to shine. It is entirely absent following short flashes, and in general is stronger the longer the exposure. This is true for the fibers giving both "on" and "off" bursts (fig. 5C) as well as for fibers giving only "off" responses (fig. 5D). Similarly the "on" burst will be absent or weak unless there has been a sufficiently long period of darkness preceding (fig. 7B). The exact time requirements for the development of the "on" and "off" bursts vary widely with different fibers.

Inhibition of the "off" response. The presence, in the vertebrate eye, of a fair percentage of fibers responding only when the light is turned off or reduced in intensity is one of the somewhat surprising findings of the present study. Although no impulses appear in such fibers during illumination, it is to be emphasized that the "off" discharge depends upon the preceding period of illumination for its excitation. The effect of this excitation does not appear until after the stimulating agent has been removed. Indeed, if the retina be re-illuminated before an "off" response has subsided, the discharge is abruptly suppressed. This may be seen in figure 5E, by comparing the effect of the brief interruption of the illumination with the fully developed "off" response following permanent cessation of the light. A very brief interruption (shorter than the latent period of the "off" response) will still give rise to a burst of impulses, but one which is very short and with considerably reduced impulse frequency. The prolonged "off" discharge following intense and prolonged illumination can always be stopped by re-illumination, even at considerably lower intensity. Following such a period of low illumination the discharge re-appears, augmented by the "off" response caused by cessation of the weak light.

This inhibitory action of light on the "off" discharge is one of the most striking features of the vertebrate optic response. Most of its effects which have been noted here have already been described in responses from the whole optic nerve (Granit and Therman, 1935; Granit and Riddell, 1934). The present results clarify the analysis considerably, by showing the rôles played by different fibers. Thus the latency of the suppression of the "off" discharge is usually very short—shorter than the latency of the "on" bursts in other fibers, especially under the conditions of light adaptation which necessarily exist. Hence in bundles containing many active fibers a brief pause occurs, shortly following re-illumination, during which no impulses are discharged (fig. 7B). This interval between the time when the activity in the "off" response fibers has been suppressed and the moment of appearance of the "on" bursts in other fibers probably corresponds to the "A" wave of the retinal action potential. As shown by Granit and Therman (1935), the principal effect of the "A" wave is to remove what "off" effect may be present; it is large when there is still a strong discharge in the "off" response fibers. When the discharge has subsided in most of these fibers, after dark adaptation, and the "on"

bursts in other fibers are stronger and have a shorter latency, the "A" wave is correspondingly small.

Stability of response types. a. *Conditions of stimulation.* It is to be seen from the preceding sections that the various types of response which are obtained in different fibers are characteristic of the particular fibers in question, rather than being due to the conditions of stimulation. Thus fibers giving "on" and "off" bursts, or only "off" responses do so over the entire range of intensities to which they respond; and fibers in which a discharge is maintained during steady illumination show this response for all intensities except near threshold. Likewise during light and dark adaptation of the eye, the type of response in any given fiber does not change. Frogs kept in bright sunlight for as long as 4–6 hours show no essential differences in the types of responses from those which have been kept in complete darkness for 48 hours, have had their eyes removed and prepared in red light, and the nerve bundles rapidly dissected in the weakest possible white light.

b. *External factors.* In order to test the possible influence of some of the more obvious external factors which might affect the retina, the following experiments were done. While not extensive, they do indicate a considerable stability in the essential features of the various response types. *Asphyxia:* if hydrogen is passed through the moist chamber, responses quickly become feeble and soon fail; they return to their former strength if oxygen is promptly readmitted. But at no time do they change their essential character during asphyxia. *pH and CO_2:* changing the pH of the Ringer's solution between 6.8 and 8.5 (phosphate buffers) with which the retina was then bathed for a few minutes produced no very apparent change in the responses, in one experiment. Two per cent CO_2 mixed with the air passed into the moist chamber causes reversible failure, with no change in the character of the responses. *Ion unbalance:* bathing the retina for a few minutes with Ringer's solution containing no calcium brings about a great increase in spontaneous activity, and tends to prolong the bursts of both the "on-off" and "off" types, with possibly some tendency to show "escape" of scattered impulses during illumination. Ringer's solution containing no potassium, on the other hand, abolishes even that spontaneous activity which may be present normally, and reduces the number of impulses in the bursts caused by change in illumination. In fibers which normally show a maintained discharge the response is not abolished, although its frequency may be reduced. These changes with unbalanced Ringer's solution are all reversible. *Temperature:* between 18° and 24°C. there is a marked speeding up of the responses with higher temperature, but there is no essential change in their character. *Season:* over a period of three years these experiments have shown no differences that could be correlated with the

season of the year, or whether the animals were freshly caught or had been kept in the laboratory.

Thus external agents which definitely affect the degree of response do not, however, change its essential character. That seems to be a fixed attribute of each particular ganglion cell.

Responses in other vertebrates. Responses in optic nerve fibers have been recorded in a variety of cold blooded vertebrates other than the frog. These experiments include one shark, one *Necturus*, a number of turtles and alligators, one iguana and several varieties of snakes. While not extensive, they show unmistakably the same general result that is found in the experiments on frogs. In the eyes of all these animals there are fibers which maintain a discharge as long as the retina is illuminated (or at least for the first several minutes), other fibers which give bursts of impulses only in response to changing the intensity of light, and still others which respond only when the illumination is reduced. Minor differences have, however, been found, and a more exhaustive comparative study might even reveal significant variation in optic function among different vertebrates. Thus in the experiment on *Necturus* the maintained discharge disappeared completely after a half minute exposure to light, and the "off" responses were feeble and required at least ten or more seconds' exposure before they could be elicited. And in both the turtle and alligator eyes fibers are not infrequently found giving only a brief burst when light is turned on, with neither maintained discharge nor "off" response. On the other hand, where one might expect to find striking differences, as in the pure cone retina of the snake, neither the actual types of response nor the relative frequency with which they were obtained differed notably from those in the mixed, though predominantly rod retina of the frog. (Only four satisfactory experiments have been done on the snake eye, since it is a difficult preparation.)

Spatial effects. No description of the optic responses in single fibers would be complete without a description of the region of the retina which must be illuminated in order to obtain a response in any given fiber. This region will be termed the receptive field of the fiber. The location of the receptive field of a given fiber is fixed; its extent, however, depends upon the intensity and size of the spot of light used to explore it, and upon the condition of adaptation; these factors must therefore be specified in describing it. For moderate intensities (less than *ca.* 4 logarithmic units above threshold) and small spots (of the order of 0.1 mm.) the receptive fields of most of the fibers of the frog's retina are roughly circular, with a diameter of the order of 1 mm. in the dark adapted condition. Even at threshold a small spot of light 0.05 mm. in diameter will usually elicit a response in most fibers anywhere within an area of *ca.* 0.5 mm. diameter. At higher intensities the size of this region from which a response can be

obtained is larger, but the strongest response is always obtained from the central portion of the receptive field; from the margins the response is usually of the threshold type (fig. 6). Thus the results of illumination of different points within a restricted region of the retina converge upon a given ganglion cell, and cause responses in its axone. This convergence extends over greater distances the stronger the stimulus; and for a given intensity the effects are strongest in the center of the region of convergence. Of particular importance to the present discussion is that, no matter what part of the receptive field is stimulated, the response in any given fiber is always essentially of the same type. This holds true for all types of response, and applies to stimulation anywhere within the receptive field. A few apparent exceptions have been observed, in which the type of response was different for different positions on the retina of the stimulating

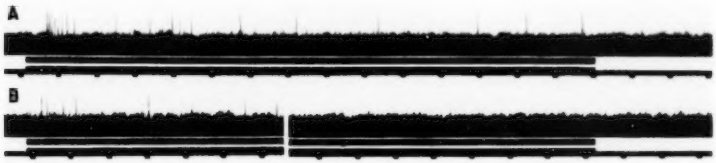

Fig. 6. Records of the impulse discharge in a single optic nerve fiber of the frog's eye, showing stronger response upon illumination in center of receptive field of the fiber than that obtained near margin.

A. Spot of light (log I = −2.0) 0.05 mm. in diameter carefully centered on most sensitive portion of the retina for this fiber.

B. Spot placed 0.22 mm. from this position (a portion of this record corresponding to 0.66 sec., and containing one impulse 0.45 sec. from the last one in the record, has been removed). Responses to this spot could be obtained anywhere within a radius of *ca.* 0.4 mm. of the position giving record A; the discharge was maintained throughout the entire period of illumination, however, only for positions very close to the central one (within ca. 0.05 mm.).

spot of light. In those cases where opportunity permitted a closer investigation, however, they proved to be either anomalous effects near threshold, or else due to two active fibers whose action potentials were so nearly similar as to be confused.[1] If true exceptions do exist, they must be quite rare; nevertheless they would be of considerable significance in explaining the diversity of the response types.

No correlation has been noted between the type of response given by a fiber and the location in the retina of its receptive field; all response types

[1] Such confusion does not arise very often, and when it does there is usually adequate opportunity to recognize the impulses due to separate fibers when the bundle begins to die, since it is very unlikely that different fibers will fail to conduct at the same time and show the same changes in the form and magnitude of their action potentials.

can be obtained from any part of the retina. (Whether this holds true for the foveal region in animals possessing a well-defined rod-free area needs to be determined.) Indeed, the receptive fields of different fibers picked up in the same bundle frequently overlap considerably, and in fortunate preparations, where impulses in different fibers can be clearly distinguished by differences in the form and magnitude of their action potentials, it is easy to show that fibers with different types of response

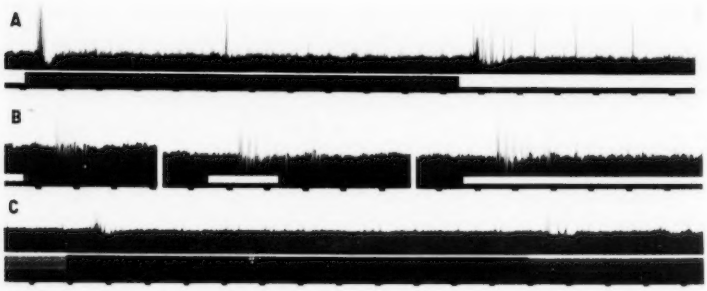

Fig. 7. Records of the discharge of impulses in bundles of optic nerve fibers of vertebrate eyes, each showing different types of responses in different fibers from the same region of the retina.

A. Bundle from turtle's eye, showing one fiber giving "on" and "off" bursts (large impulses); another discharging impulses throughout whole period of illumination (small impulses); and a third giving only a brief "off" response (medium sized impulses).

B. Bundle from frog's eye, showing two active fibers, one giving typical "on" and "off" bursts (large impulses), the other responding only to turning light off. Reillumination after 0.36 sec. (middle part of record) stopped the discharge in fiber giving only the "off" response (compare with last part of record, where light was left off) and produced a very weak "on" response in the "on-off" fiber.

C. Bundle from frog's eye, showing a fiber (small impulses) which maintained its discharge at a low frequency during period of illumination at beginning of record. Intensity doubled, this fiber gave an initial burst and then maintained its discharge at a higher level of frequency. Intensity reduced to its former value; frequency in this fiber dropped, and another fiber responded with a vigorous "off" response, (large, thin impulses). A third fiber (medium sized, thin impulses) gave a short burst in response to the increase, and very few impulses (difficult to distinguish) in response to the decrease.

are usually present and are stimulated simultaneously by a small spot of light (fig. 7). Such observations constitute good evidence for the distinctness of the response types, for they are obtained under identical conditions of adaptation, stimulation, and external environment.

Discussion. From this experimental study it is apparent that each individual ganglion cell has a relatively fixed character of response, which, with few exceptions, falls under one of three distinct types. Concerning

the explanation of this rather unexpected result one can only speculate. The retina, of course, does not possess a homogeneous population of end-organs, and it is possible that the different types of response originate in different types of visual sense cells, merely being relayed through the ganglion cells unaltered. Thus the rods and cones might give rise to responses of different types. Ignoring for the moment the presence of all three types of response in the pure cone retina of the snake, it is still difficult to fit three response types into a duplicity theory, and evidence is still lacking that would associate any of the response types with either rod or cone function. But different forms of both rods and cones are present in most cold-blooded vertebrate retinas, and different sense cells show adaptation of their responses in different degrees. It is not unreasonable to assume that certain of either the rods or cones, or both, might adapt completely following their initial discharge of impulses. Even pure "off" responses might be supposed to originate in certain cells excited by a shift in their equilibrium in the sense opposite to that which usually gives rise to the discharge of impulses.

The diversity of response might, on the other hand, originate in the layers of the retina between the rod and cone layer and the ganglion cells. Thus a given ganglion cell may be subject to diverse and rival influences, and its response determined by the relative amounts of each. This, in turn, may be fixed in large measure by the anatomical connections between the ganglion cell and its underlying neurones. This study in fact has shown clearly that the excitation produced by light may be subject to modification by an influence which may justifiably be termed inhibitory. Thus the relative amounts, and rates of rise and fall of excitatory and inhibitory influences upon a ganglion cell might determine its response. The "off" effect may then be due to a post-inhibitory release of the effects of an excitation which are all the greater for having been suppressed during the actual period of illumination. Granit and his co-workers have urged the use of these concepts in the interpretation of optic response.

Still another explanation to be considered is the possibility that functional differences may exist among the ganglion cells. While subject to essentially the same influences from the underlying retinal layers, different ganglion cells may respond differently to shifts in their equilibrium. The further consideration of these and other possibilities must wait upon the results of further experiments.

SUMMARY

1. Action potentials in single optic nerve fibers of cold-blooded vertebrate eyes may be obtained from small intraocular bundles dissected off the anterior surface of the retina of excised, opened bulbs.

2. Responses in different single fibers from the same retina show differ-

ent types of response. In about 20 per cent of the fibers response to illumination of the retina begins with a burst of impulses at high frequency, followed by a steady discharge at lower frequency which is maintained throughout illumination, and stops when the light is turned off. About 50 per cent of the fibers show only a burst of impulses in response to the onset of illumination, and another in response to its cessation; no impulses are discharged during steady illumination of the retina. The third type of response, obtained from about 30 per cent of the fibers, shows no discharge either at the onset of illumination, or throughout its duration, but gives a vigorous and prolonged discharge when the light is turned off.

3. In general the higher the intensity of retinal illumination the shorter is the latent period of the response, the higher its frequency of discharge, and the greater the number of impulses in a burst.

4. Fibers which give a response to the onset and cessation of light also respond, though less strongly, to an increase or a reduction in its intensity. Fibers giving only an "off" response respond, though less strongly, to a reduction in intensity; they give no response to an increase.

5. As dark adaptation of the eye progresses, the discharge which can be obtained from any fiber in response to a given intensity increases; the threshold of response correspondingly falls, rapidly at first and then more gradually, for half an hour or more.

6. "Off" responses are weak or absent following short periods of illumination; similarly "on" responses require a sufficiently long preceding period of darkness for their full development.

7. The discharge in fibers giving only an "off" response is promptly suppressed by re-illumination of the retina.

8. The type of response in any given fiber does not depend upon conditions of stimulation or adaptation of the eye. Even certain external agents (asphyxia, CO_2, ion unbalance, temperature), while affecting the responses do not alter their essential character.

9. Experiments on fish, amphibian and reptilian eyes give essentially the same results as regards the types of response found.

10. Responses can be obtained in a given optic nerve fiber only upon illumination of a certain restricted region of the retina, termed the receptive field of the fiber.

11. The location on the retina of the receptive field of a fiber is fixed. Its extent depends upon the size and intensity of the spot of light used to explore it, and upon the state of adaptation of the eye.

12. With possible rare exceptions the type of response in any fiber does not depend upon the portion of its receptive field which is illuminated.

13. The type of response in a fiber is not correlated with the location of its receptive field in the retina.

14. The question is discussed as to whether the diversity in types of

response is due to different types of sensory cells, whether it arises in the intermediate layers of the retina, or whether it is the result of functional differences among the ganglion cells.

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THE NATURE OF MAGNESIUM TETANY[1]

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Symptoms and sequelae. The condition of hyperexcitability induced by magnesium deprivation was first observed by Kruse, Orent and McCollum (1). In their opinion this condition represents a new form of tetany distinguished by a normal blood calcium and an absence of alkalosis. A study of the syndrome of magnesium tetany has been made in the course of the present work with results similar to those of Kruse, Orent and McCollum. However, it is of interest to describe certain differences which were noted and the type of variability introduced into the syndrome by changing magnesium levels and certain other factors in the diet.

The diets and the experimental procedures employed in this work are described in the first paper of this series (2).

Peripheral vasodilatation. The high degree of constancy in the time of appearance and the duration of the vasodilatation and hyperemia observed by Kruse, Orent and McCollum were not found with the diets employed in this work. At a dietary level of 0.4 mgm. of Mg per 100 grams food, vasodilatation appeared in all the animals in about seven to nine days and persisted for a period of from a few hours up to four days. Above this magnesium level, the percentage of animals which were affected decreased and even greater variation appeared in the time of onset and duration of the phenomenon. With 2 mgm. per cent magnesium in the diet, vasodilatation appeared in only half the animals and the average time of onset was at about twenty-one days. With a diet containing 5 mgm. per cent Mg and 0.87 per cent Ca, approximately two-thirds of the animals exhibited redness of exposed skin areas from the fifth to the eleventh day, then it subsided and reappeared again for one or two days on about the thirtieth day of the experiment. Occasional reappearance of the hyperemia has also been observed in older animals by Kruse, Orent and McCollum. In animals fed a 5 mgm. per cent Mg diet containing 1.16 per cent Ca, vasodilatation appeared as early as the third or fourth day in a very severe form and lasted four to five days. Variation in the age of the animals at the start of magnesium depletion seemed to have little effect on the appearance of this phenomenon.

[1] This work was aided by grants from the Christine Breon Fund for Medical Research and the Division of Natural Sciences of the Rockefeller Foundation.

Handling of the animals or taking them to a cooler environment cause the vivid scarlet color which is particularly conspicuous in the ears to fade rapidly. The deep color returns in a short while after the animals are returned to their cages.

The symptom of vasodilatation varied not only in the duration and time of its appearance, but also in its time relationship to the appearance of hyperexcitability. On diets containing less than 1 mgm. per cent of magnesium it appeared concomitantly with the first symptoms of hyperexcitability. On 1.0 to 1.5 mgm. Mg diets it was possible to produce convulsions three to four days after the appearance of vasodilatation and on 1.8 to 2.2 mgm. Mg diets, eight to twenty days after its appearance. In a few cases convulsions were induced before the onset of vasodilatation.

Hyperexcitability. The effect of varying the magnesium content of the diet on the time elapsing before the first convulsions is shown in figure 1. The results are much what one would expect. At levels of less than 1 mgm. of Mg per 100 grams food all experimental animals reacted within 10 to 14 days and their total life span was from 21 to 30 days with death resulting from a spontaneous convulsion. At levels of about 1.2 mgm. of Mg per 100 grams food, 90 per cent of the animals reacted at an average time of fourteen days and their life span was somewhat increased. At a magnesium level of 1.5 mgm. the onset of hyperexcitability took place at about twenty-two days and involved three-quarters of the rats. Some of the animals survived as many as three attacks of convulsions and the life span was increased 35 to 47 days for the majority of the animals. At a 2 mgm. level the time of onset of hyperirritability, on an average, is postponed until the thirty-third day, and involves only about half the animals. The life span is increased almost indefinitely and the animals on this diet have been known to survive as many as five convulsive attacks. With the low calcium, 5 mgm. per cent Mg diet, there was usually some evidence of hyperexcitability in about one-quarter of the animals after two to three weeks on the diet. The animals then became resistant for an indefinite period of time, when a second onset of excitability occurred in most of the animals. Two animals which had had no previous convulsions on their record were found to be hyperexcitable during lactation at 157 and 200 days on the diet, respectively. Another animal with a record of a convulsion at nineteen days on the diet was shown to be hyperexcitable after 126 days on the diet. No signs of hyperexcitability were observed in rats receiving more than 5 mgm. Mg per 100 grams of diet unless the calcium content was augmented.

Aside from the magnesium level, the vitamin G complex and calcium contents of the diet and the initial weight of the animals were found to have a marked effect on the time elapsing before convulsions could first be induced. The influence of the first two factors becomes more apparent

when the deficiency is not too severe. With regard to the last factor, within certain limits, the time elapsing before the appearance of hyperirritability increased in proportion to the weight of the animal.

Our attention was turned to the vitamin G complex as a possible factor in the picture of magnesium deficiency because it was observed that both

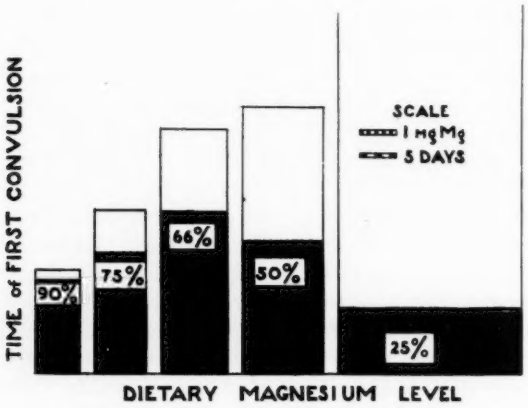

Fig. 1. Relation of magnesium intake to the experimental period elapsing before the first induced convulsion. The height of the entire column along the ordinate represents the number of days after the beginning of depletion at which the first convulsion was observed. The width of the base of each column along the abscissa is equal to the magnesium intake in milligrams Mg per 100 grams food. The shaded portion of the column indicated the proportion of the animals which became hyperexcitable at the time mentioned above. From left to right, the data may be summarized as follows:

	mgm. Mg in diet	*Number of animals*
Column I	1.2	12
Column II	1.5	25
Column III	1.8	12
Column IV	2.2	6
Column V	5.0	11

The starting weight in *all* groups shown in this figure was between 50 and 60 grams, and the vitamin G and B intake was uniformly in excess of twice the amount necessary for optimum growth.

the control and deficient animals show signs of malnutrition when the water soluble vitamin supplement was given in the form of an alcoholic extract of yeast, prepared according to the directions of Kruse, Orent, and McCollum.[2] All symptoms of the deficiency were alleviated in the

[2] From yeast powder purchased from the Northwestern Yeast Company.

control animals, but not in the magnesium low animals by feeding additional vitamin G in the form of liver extract.

The following series of experiments illustrate the influence of vitamin G on the onset on magnesium tetany. On a diet of 2.0 mgm. per cent Mg, one group of deficient animals was first depleted of vitamin G and then fed an amount necessary to make the corresponding controls gain 10 to 15 grams a week. A second group was fed from the start and throughout the fifteen weeks of the experiment a vitamin G level necessary for a gain of 10 to 15 grams a week. The third group was fed vitamin G in excess of the amount necessary for optimum growth. Two-thirds of the animals in the first group became hyperexcitable and convulsions were induced at times varying from the fourth to the twenty-first day. Half of the second group and only a third of the last group became hyperexcitable. In the latter two cases the first convulsions appeared about the thirty-third and fortieth day, respectively. This phenomenon was not related to any difference in magnesium depletion as shown by subsequent analysis, nor was the hyperexcitability developed in the first group related to the vitamin G depletion alone, since controls at this same vitamin G level showed no symptoms of hyperexcitability. Probably the explanation of the apparent synergism of the two deficiencies lies in the fact that vitamin G deficiency itself promoted some damage to the nervous system which would tend to increase its reactivity so that the effects of the two deficiencies are additive.

The effect of increasing the calcium level of the diet is to shorten the average time of onset of irritability and to increase the incidence and the severity of the convulsions. Thus, only about a quarter of the animals of the 5 mgm. Mg low calcium diet became hyperexcitable and all survived convulsions, while two-thirds of the animals on the 5 mgm. Mg high calcium diet showed the first signs of hyperexcitability in 11 to 32 days and succumbed to fatal convulsions in from 16 to 72 days.

The effectiveness of different types of stimuli in producing convulsions is of singular interest. We have attempted to use visual, electrical, and auditory stimuli for inducing convulsions. The visual stimulus of short flashes of a powerful light was entirely ineffective. Shocks from an induction coil were ineffective in all but one or two cases, although they frequently produced some signs of hyperexcitability in the animal. Several types of auditory stimuli have been tried, namely, a short sharp noise produced by firing a cap pistol, various tones through the audible range produced by an oscillator, the hissing of an air blast, and the sound of rapidly running water. Only the last two stimuli threw deficient animals into convulsions. The sound of an air blast is the most potent and as the animals become more and more deficient a shorter and softer stimulus is sufficient to throw them into a convulsion. The sound of rapidly running

water is effective only for extremely deficient animals. There was apparently no adaptation to the air blast sound since it did not require greater stimuli to induce convulsions in rats previously exposed to the stimulus when unexcitable, or in those which had had previous convulsions. Apparently the deficiency produces a far greater sensitivity in the auditory system than in the peripheral, sensory, muscular, or visual systems. Furthermore, the sensitivity must involve a fairly specific part of this system since only two very similar sounds can be considered as effective stimuli. This is very significant in the light of the effect on this condition of certain drugs which are described below.

Site of the lesion. Protection against convulsive attacks is not afforded by the injection of a magnesium salt (1 to 2 mgm. Mg intraperitoneally) for a period of up to four or five hours following the injection. This observation does not harmonize with a view that the hyperirritability in this condition is a peripheral affection or is merely the result of an ion imbalance in the body fluids.

In order to obtain more information on the location of the lesion in the nervous system responsible for the hyperirritability in magnesium deficiency, experiments were carried out with certain drugs which are generally agreed to have their principle and characteristic actions in specific regions of the central nervous system. Contrasting tests were made on the sensitivity of hyperirritable magnesium deficient and of control rats to the convulsant action of strychnine sulphate, picrotoxin, and cocaine hydrochloride. The convulsant action of these drugs is stated to be centered in the spinal, midbrain, and cortical region, respectively (3). No differences in the size of the dose required to produce convulsions were found with either strychnine or cocaine. On the other hand, the magnesium deficient animals appear to have a small but definitely increased sensitivity to the convulsant action of the midbrain drug, picrotoxin. This is shown by the data given in table 1.

It will be noted that at a level of 0.25 mgm. of picrotoxin per 100 grams of body weight, some 95 per cent of the deficient rats tested succumbed to convulsions, nearly 50 per cent being fatal. At this dosage none of the controls succumbed and only 25 per cent showed onset of a convulsion. From table 1 it would appear that a dosage of more than 0.35 mgm. per 100 grams of body weight is required to produce an equivalent effect on the controls. There appears to be a definitely increased sensitivity to picrotoxin in this condition, although the magnitude of the difference is not extremely great.

If the site of the hyperirritability is situated in the central nervous system, magnesium tetany is of a definitely different type than is parathyroid or low calcium tetany. Considerable evidence exists that the lesion in parathyroid tetany is located in the neuro-muscular junction.

This is shown, for example, by the antidotal effect of curare on this condition as has been demonstrated by Hartridge and West (4). Curare, we have found, does not protect against, but instead increases the severity of the convulsions in magnesium tetany. This indicates that the causes of these two conditions are not similarly situated. The effect of curare in magnesium tetany is shown in table 2. In these experiments, convulsions were induced by the usual stimulus used to test hyperirritability in magnesium deficiency, namely, the hissing sound of an air blast. The curare dosages employed were below the level which produces paralysis. It was

TABLE 1

Increased sensitivity of magnesium deficient rats to the convulsant action of picrotoxin

	DOSE	FATAL CONVULSION	NON-FATAL CONVULSION	NO CONVULSION	NUMBER AFFECTED
	mgm. per 100 gram rat				*per cent*
Deficient	0.25 0.35 0.50	10	11	1	95
Controls	0.25	0	5	15	26
	0.35	1	4	1	84
	0.50	0	3	3	50

TABLE 2

*The effect of curare on the incidence of convulsions in magnesium deficient animals**

DOSE	NUMBER HAVING CONVULSIONS	NO CONVULSIONS
mgm. per 100 gram rat		
0.25	5	1
0.15	4	1

* The curare solution was prepared by steeping the weighed material overnight in warm saline solution. Since a small portion did not dissolve, the dosages given in the table are only approximate.

determined on control animals that a fatal paralysis develops at a dosage of about 0.5 mgm. of curare per 100 grams of rat.

The curarized deficient animals suffered more severe convulsions than is the case for non-curarized animals. In practically all cases, the curarized animals died of respiratory failure in the course of the convulsion, probably because of the weakening effect of the curare. The two rats listed in table 2 which gave a negative response were probably not sufficiently hyperirritable to be at the convulsive stage.

Further evidence favorable to the view that the lesion responsible for the hyperirritability in magnesium deficiency is situated in the region of

the midbrain is offered by the action of the barbiturates in protecting against convulsions in this condition. It has been shown by Swanson and Chen (5) that sodium amytal affords protection against the convulsive effect of the midbrain drugs, picrotoxin and cori-amytrin. Similarly, sodium amytal is an antidote against and will prevent the onset of convulsions in magnesium deficiency. In an experiment to demonstrate this, a dozen deficient animals which had previously been shown to be hyperirritable were injected intraperitoneally with sodium amytal in amounts of 2.5 and 5.0 mgm. per 100 grams of body weight. This is, respectively, about $\frac{1}{3}$ and $\frac{2}{3}$ the anesthetic level of this drug in the rat. These animals were completely resistant against attempts to induce convulsive seizures for a period of about five hours after injection.

On the basis of the experimental evidence given in this section, it may be suggested as a tentative hypothesis that the hyperirritability in magnesium deficiency is associated with an affection located in the midbrain or pons and in particular seems to involve the central reflex connections of the cochlear nerve.

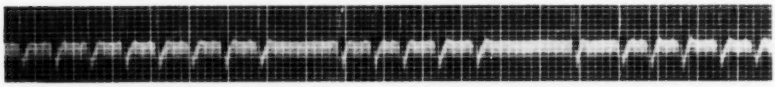

Fig. 2. Electrocardiogram of a rat taken during a magnesium tetany convulsion.

The convulsions in magnesium tetany, whether they are induced or spontaneous, always follow more or less closely the pattern outlined by Kruse, Orent, and McCollum (1). The animals may exhibit some signs of hyperirritability before any convulsions are observed, i.e., they are more easily frightened by noises or sudden movements than the controls, run restlessly about their cages, and occasionally bite the cages violently. Frequently a pronounced protrusion of the eyeballs appears for the two or three days previous to a convulsion. This exopthalmos has been mentioned by Day, Kruse, and McCollum (6) as being a symptom of a compound magnesium and calcium deficiency. The exophthalmos becomes progressively worse until after the seizure when it subsides. The convulsive attacks vary greatly in severity and the animals may remain in a rigid unconscious state for only a moment, or, in a very severe attack, for perhaps fifteen minutes. In a few instances, animals have been observed which did not become unconscious during the excited state, but ran in circles about the cage, bit the cage or other rats in it, and emitted sharp squeaks. This condition usually lasts much longer than the excited state in which a convulsion actually takes place. In contrast to the above authors, we have never observed priapism during convulsive seizures

in any of our animals. The recovery period covers a much longer time than the convulsion and may occupy almost two hours.

Magnesium deprivation causes an increase in the pulse rate. Electrocardiographic records of rats under light amytal anesthesia showed that the heart rate of deficient animals, on a 1.2 mgm. per cent Mg diet for 5 days, averages about 20 more beats per minute than the controls, and 40 beats per minute more between the period of 10 to 40 days on the diet. A convulsive seizure drastically slows the heart and leads to an arrhythmia. Electrocardiograms taken of rats while unconscious from convulsive seizures show evidence of a sino-auricular block. The sinus beat is rhythmic, but both auricles and ventricles skip beats from time to time (see fig. 2).

We are indebted to Dr. Robert Aird for much helpful advice in the neurological aspects of this work, and to Dr. Eric G. Ogden for assistance in taking the electrocardiograms.

SUMMARY

1. A study has been made on the influence of a number of factors on the incidence, time of onset, and duration of peripheral vasodilatation and hyperirritability in magnesium deficient rats. These symptoms were found to be greatly affected by the degree of magnesium deficiency, the starting age of the rats, and the dietary levels of calcium and vitamin G.

2. The localization of the lesion concerned with the hyperirritability appears to be in the midbrain or pons. This is supported by an increased sensitivity of the deficient animals to the convulsant action of picrotoxin and the protective action of sub-anesthetic doses of sodium amytal against convulsions.

3. Magnesium tetany differs from calcium tetany in that curare does not prevent the onset of convulsive seizures in this condition.

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THE EFFECT OF MAGNESIUM DEPRIVATION ON RENAL FUNCTION[1, 2]

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The condition of hyperexcitability and trophic changes in the epidermic structures of the rat brought about by magnesium deprivation were first observed by Kruse, Orent and McCollum (1).

Cramer (2), in 1932, found that most of the rats which he placed on a prepared synthetic diet low in magnesium (stated to contain 0.632 mgm. per 100 grams of food) developed marked changes in the kidneys. At autopsy, the kidneys of many of these animals were found to be enlarged and had puckered surfaces, some showing a gray mottling. On cut section, they exhibited definite striations at the junction of the medulla and cortex.

Histologic preparations presented a picture of extensive degeneration of tubules and glomeruli, which was most marked at the junction of the medulla and cortex where, in some of the sections, the necrosis was accompanied by calcareous deposits in the tubules and glomeruli.

These observations were rendered considerably less significant by the fact that the kidneys in about half of the animals of the control groups also exhibited some degeneration which was confined to the junction between the cortex and medulla. The lesions in the control animals were characterized by calcifications in the tubules. This abnormality was attributed to an excess in the diet of vitamin D, which was furnished by cod-liver oil.

Cramer stated that over a period of six weeks his experimental animals grew as rapidly and looked as well as did the rats given the control diet. With so low a level of magnesium in the diet as was given, it is difficult to understand the absence of symptoms of hyperexcitability or of nutritional deficiency.

Brookfield (3), working with rats and using the diet of Kruse, Orent and McCollum, observed similar changes in the kidneys of animals given a diet of low magnesium content. He observed also degenerative changes which involved the Kupffer cells in the liver. This latter finding has not

[1] This work was aided by grants from the Christine Breon Fund for Medical Research and the Division of Natural Sciences of the Rockefeller Foundation.

[2] A preliminary report has been published in J. Biol. Chem. Proc. **114:** xliii, 1936.

been confirmed by us. Like Cramer, Brookfield observed no symptoms of nutritional deficiency in his animals. He states, however, that both groups of animals, those receiving the control diet and those receiving the magnesium-deficient diet ceased to grow after a short period of time. From the results of our work, it would appear that the basic diet used by Brookfield may have been low in one of the vitamin G factors, and it is possible that a deficiency of some water-soluble vitamin component caused cessation of growth in both the control and the experimental groups.

MacKay and Oliver (4), by feeding animals on a diet containing excessive amounts of inorganic phosphate, produced lesions in the kidneys comparable in their general features to those resulting from magnesium deprivation. These authors stated that the salt mixture used by Cramer contained a sufficient amount of phosphate to produce renal damage; and, further, that they were unable to produce the lesions solely by means of a diet low in magnesium.

In view of these conflicting reports, it has been considered desirable to publish the results of our series of observations on the changes in renal function that occur in rats reared on diets low in magnesium.[3]

In a previous paper of this series (5), it has been shown that the course of magnesium deficiency in the rat consists of two fairly definite phases: the first characterized chiefly by vasodilatation, hyperemia and hyperexcitability; and the second by signs and symptoms of nutritional deficiency and renal damage. In the course of the second phase of magnesium deficiency, the alterations found in a number of bodily functions lead to the conclusion that prolonged magnesium deprivation eventually causes a nephrosis-like type of kidney degeneration. This conclusion is supported by the following findings; first, definite changes in the kidneys were found similar to those first observed by Cramer, except that the degree of calcium deposition was greater in our experiments. Second, the blood serum proteins were definitely lowered. (In the final stages of deficiency the protein levels have, in some instances, been so low that they could easily account for the edema which appeared.) Third, the deficient animals showed an increased excretion of protein nitrogen, a persistent albuminuria, and an increased volume of urine. No increase of casts was observed, and tests for the presence of blood in the urine were consistently negative. These findings are compatible with those observed in the nephrotic types of renal disease.

The control animals, fed the same diet (containing 0.87 per cent calcium) and a supplement of 50 mgm. of magnesium per 100 grams of food, showed no evidence of gross or histologic changes in the kidneys.

From a study of the microscopic sections of the kidneys of the animals

[3] The composition of the diets and the experimental procedures which have been employed are reported in reference (5).

sacrificed, the renal changes occurring during successive stages of magnesium deficiency may be described as follows:

General appearance. With a mild degree of deficiency, diffuse hemorrhage and engorgement of the vessels of the medulla occur. In the advanced stages of deficiency, the kidneys are enlarged and edematous. In most cases the capsule is found to be thin. In the cortex the tubules are prominent. Some contain hyaline material and others are surrounded by round-cells. There is some granular debris and a few concretions of calcium in the tubules.

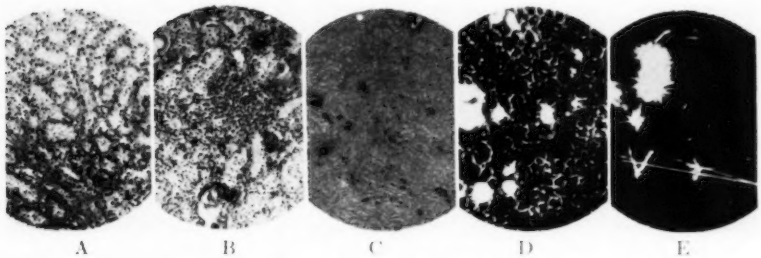
Cortico-medullary boundary zone. This is the region of greatest calcification. In the initial stages the tubules are seen to contain much granular and pultaceous debris. At a more advanced stage of deficiency, large concretions of calcium occur in the outer stripe of the boundary zone, crowding the adjacent tubules. The tubules containing calcium become distended, and the epithelium atrophies. There is no evidence of inflammatory reaction. The calcium deposits extend throughout the medulla but are progressively smaller as one approaches the tips of the pyramids. Practically all the convoluted tubules of the area contain calcareous material in various stages of condensation.

Medulla. In most instances, many small concretions of calcium may be seen in the medulla. Around the concretions are patches of diffuse tubular atrophy. In some of the tubules there is a collection of amorphous material and others contain pink hyaline material. A prominent finding in the medulla is the increase in interstitial connective tissue, best seen with von Giesson's stain. With the von Kossa stain, the concretions in the boundary zone and the medulla are stained brown.

Illustrations of the findings indicating renal damage resulting from deficiency of magnesium are shown in the photomicrographs in figure 1.

Proof of the calcareous nature of the deposits that were observed in these kidneys has been obtained in a number of ways: First, the reaction to the von Kossa stain was positive (fig. 1-C). Second, microincineration of sections of kidney demonstrated large mineralized areas (fig. 1-D). Third, on addition of sulfuric acid to the incinerated sections, gypsum crystals formed at the periphery of the residual masses of mineral matter. Fourth, there is an increase in the quantity of calcium found in the kidney (see table 2).

Besides histologic changes, the kidneys showed also such gross changes as light-colored patches or pitting of the surface of the kidney, and irregular coloring in the cortex of fresh sections. In general, the kidneys of the deficient animals were not greatly enlarged. The kidneys of animals in a more advanced stage of magnesium deficiency were heavier in terms of unit of body-weight than were those of the control animals. However, since the deficient animals became emaciated and retarded in growth,



A B C D E

Fig. 1. Photomicrographs illustrating nephrophathic changes produced by magnesium deficiency. (Reproduced one-half original size.)

A. Microscopic section of kidney of control animal. × 125. Hematoxylin and eosin stain.

B. Microscopic section of kidney of deficient animal. × 125. Hematoxylin and eosin stain. The dark staining areas are calcareous casts.

C. Von Kossa stained section of kidney of deficient animal. × 50. This section illustrates the extensive calcareous deposits, which are found in the cortico medullary region following prolonged magnesium deprivation.

D. Micro-incinerated section of the kidney of deficient animal. × 125. This shows the mineral matter of the large calcified areas left after incineration.

E. This photomicrograph gives proof of the calcareous nature of the casts observed in the kidneys of magnesium deficient animals. Gypsum crystals were formed when dilute sulfuric acid was added to the incinerated section. The crystals are shown in the photograph.

TABLE 1

Comparison of the urine of magnesium deficient and control rats

	CONTROL TOTAL VOLUME OF URINE = 21.1 ML.						DEFICIENT TOTAL VOLUME OF URINE = 49.9 ML.					
	Days											
	1	2	3	4	5	6	1	2	3	4	5	6
Volume	3.3	2.0	1.5	4.4	5.7	4.2	9.9	7.5	7.5	8.5	12.0	4.5
Albumin	−	−	−	−	−	−	+++	++	++	+++	+++	+++
pH	8.0	8.2	8.2	8.1	7.8	7.2	8.9	8.9	8.9	8.9	8.4	8.3
	Total protein N 380.0 mgm. for the 6 day experimental period						Total protein N 1010.0 mgm. for the 6 day experimental period					

The experiment was carried out on groups of 3 animals of 65 grams starting weight which had been maintained for 2 weeks prior on a diet of 1.5 mgm. of magnesium per 100 grams food.

it is questionable as to whether the greater weight represented hypertrophy of the kidney.

As the deficiency was prolonged, there was an increased excretion of protein in the urine, and a further increase in urinary volume. The

figures given in table 1 were obtained for urine collected over a period of six days from three control and three deficient animals.

The content of protein in the blood serum began to fall off with the beginning of the second phase of deficiency. As early as the thirtieth day, rats fed on a diet containing 2.0 mgm. per cent of magnesium began to show serum protein values just below the normal range (varying from 5.1 to 6.0 per cent). As the second phase of deficiency progressed, the protein content of the blood serum continued to fall, reaching levels considerably below the normal range. The average final value obtained for these rats was 4.9 per cent, with a range of 3.3 to 6.0 per cent. When the rats had been on the diet for eighty-one days, a total blood serum protein of 4.25 per cent, with an albumin value of 2.25 per cent was observed. The incidence of low protein in the blood serum was associated with the appearance of edema, and it is reasonable to suppose that the

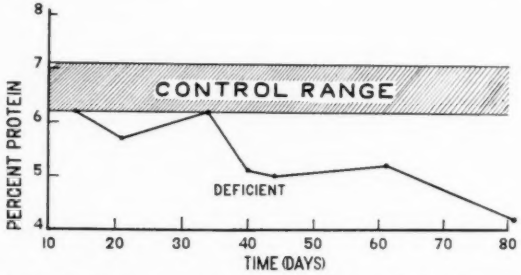


Fig. 2. Variation in the serum protein level with time during magnesium depletion of rats on a diet of 2.0 mgm. of magnesium per 100 grams of food. The serum protein range of normal control animals is indicated in the cross hatched section.

edema developed as the result of protein depletion. Results of serum protein determinations on these animals are presented graphically in figure 2.

Discussion. Extensive renal damage was observed in rats kept on a diet containing 2.0 mgm. per cent magnesium for fifteen weeks, and likewise in rats kept on a high calcium diet (1.16 per cent of calcium), containing 5.0 mgm. per cent of magnesium. Severe renal damage was observed in rats fed a diet containing 1.0 mgm. per cent of magnesium and kept alive for six weeks by occasional injections of smaller amounts of magnesium sulfate. Rats fed diets containing less than 1.0 mgm. per cent magnesium usually lived only twenty-five to thirty days; in these animals, renal damage was observed in a smaller number of cases and in a less severe form than in animals given a somewhat larger allowance of magnesium and who therefore lived a longer period of time. When the diet contained 5.0 mgm. of magnesium per 100 grams of food with a moderate calcium

content (0.87 per cent calcium), the pathologic changes observed in the kidneys were similar to those noted during the early phases of magnesium deficiency. It is possible, however, that a longer period of depletion with a diet slightly deficient in magnesium would produce equally severe changes in the kidney.

Whereas hyperexcitability is a symptom of acute magnesium deficiency, the type of renal damage observed is the result of chronic magnesium deficiency. It is not certain what part the nephrosis-like condition and attendant loss of protein plays in the production of the degree of malnutrition characteristic of the later stages of deficiency. In the early stage of the second phase of magnesium deficiency, anorexia is responsible for the malnutrition; as magnesium deficiency continues, the kidney becomes progressively damaged, and there is loss of protein in the urine with subsequent diminution of blood serum protein. This accentuates

TABLE 2

Showing calcium accumulation in the kidney in magnesium deficiency

NUMBER OF ANIMALS	CONTROL GROUP CALCIUM IN FRESH KIDNEY		NUMBER OF ANIMALS	DEFICIENCY GROUP (CALCIUM IN FRESH KIDNEY)	
	Range	Mean		Range	Mean
	mgm. per cent	*mgm. per cent*		*mgm. per cent*	*mgm. per cent*
17	6.9–12.4	9.4	22	48.5–232	141

the picture of malnutrition, and edema develops. The final stages of cachexia and edema are therefore due to the pathologic changes induced in the kidney.

The phosphate content of the diet can hardly be a factor in our experimental results since the total phosphorus in the diets employed varied from 0.45 to 0.8 per cent. The renal damage in deficient animals appeared to be independent of the amount of phosphorus in the diet; and the kidneys of the control animals were normal regardless of the content of phosphorus in the diet.[4]

In view of the evidence obtained by special staining of the sections of kidney for calcareous deposits and the increased calcium content of this organ (table 2), it appears that calcification played an important part in the degeneration of the kidneys although it may have been preceded

[4] In experiments carried out with a magnesium deficient diet relatively high in calcium (1.66 per cent) kidney damage of a milder degree than in the deficients was observed in the control animals. Since this diet contains 1.0 per cent phosphorus, which is a level at which MacKay and Oliver obtained renal damage, it is uncertain whether the high calcium or phosphorus content of the diet is at fault in this respect. Experiments are now under way to determine whether high calcium diets, per se, are nephropathic.

by other degenerative changes. It has been shown that solutions low in magnesium favor the "in vitro" calcification of bone and that magnesium acts in some specific manner to increase the solubility of calcium salts (6), (7). The calcification taking place in the kidney can be partially explained by this effect. However, the actual mechanism of this solubility-effect is not known, and it seems very likely that local factors such as tissue damage also contributed to the calcification taking place in the kidneys of magnesium deficient rats.

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SUMMARY

1. Prolonged deprivation of magnesium eventually produces pathologic degeneration in the kidneys which, in certain respects, is similar to that found in nephrosis.

2. There is an increase in volume of urine and in excretion of protein, but no blood or casts are present in the urine.

3. The blood serum protein content undergoes a progressive diminution, finally reaching levels at which edema develops.

4. The pathologic changes in the kidney include calcification in the cortico-medullary zone, in the pyramids, and at a later stage in the cortex. In the earlier stages, degenerative changes in the tubules are a more constant finding than is calcification.

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COMPARATIVE OBSERVATIONS ON MOTOR AND SENSORY FIBERS WITH SPECIAL REFERENCE TO REPETITIOUSNESS[1]

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While experimenting with spinal root preparations of the bullfrog it was found that during the passage of a constant current a repetitive response was obtained more commonly from the sensory than from the motor fibers. The present paper presents evidence indicating that this difference is characteristic, and also experiments having as their end attempts to disclose other differences between motor and sensory fibers, in particular such as might prove to be of interest relative to the difference in repetitiousness.

PREPARATION AND METHODS IN GENERAL. The root pairs (usually the VIIIth or IXth, the VIIth occasionally), together with the trunk formed by their union, were studied either immediately upon removal or after refrigeration in Ringer's solution. In the refrigerator irritability was retained for as long as two weeks, and responses to all appearances continued normal. The preparations were suspended vertically in the moist chamber, under the tension of their own weight, from threads tied to the root ends. The roots deviated from the vertical only by enough to keep them apart on their respective electrodes. The positions of all supports and electrodes were adjustable from without the moist chamber. The electrodes were of silver excepting when used to convey constant current; then in most of the experiments calomel half-cells were employed. Except where otherwise stated, stimuli were break shocks from coreless Porter coils. The methods of expressing shock and constant current strengths are those described in previous publications (Erlanger and Blair, 1936; Erlanger and Gasser, 1937). There will be found also descriptions of our amplifying and recording techniques.

The root preparation is peculiarly suited to a comparative study of the properties of motor and sensory fibers, since in the trunk the two kinds of fibers are exposed to the same conditions, while from the roots their conducted responses can be recorded separately. A comparison can also be

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made through stimulation of the roots individually while leading from the trunk, though not so unequivocally, since with this arrangement the results are conditioned by any differences there may be in electrode contacts and in the diameters of the two roots. Electrode positions were read by means of a cathetometer. Stimulation was taken to occur at the edge of the cathode proximal to the anode; and an action potential coursing along the preparation was taken to record as it leaves the grounded lead on its way to the grid lead on the killed end.

In interpreting the data derived from this preparation certain structural features must be taken into consideration. The roots are devoid of epineurium. The epineurium of the trunk is continuous with the dura mater in the region of the ganglion (Tarlov, 1937), and to remove the preparation the adherent dura must be cut away from the ganglion. Occasionally in this process the latter is nicked slightly by the scissors. The motor root joins the sensory either at the ganglion or just distal to it; in the absence of definite information, it is assumed that the motor and sensory fibers are uniformly mixed in the trunk 5 mm. beyond the ganglion. Excepting the dorsal ramus, which is given off at the peripheral edge of the ganglion, and the rami communicantes, which arise from the middle section of the trunk, these preparations are essentially branchless. However, it must be borne in mind that where these branches have been cut, and also in the vicinity of the injured ends of the preparation, demarcation currents can develop which could modify induced responses.

Results. *Motor and sensory rheobases along the preparation.* In the course of these experiments considerable information has been acquired regarding the constant current excitability of the motor and of the sensory fibers along the length of these preparations. This mapping of constant current excitability (and also of repetitiousness) is accomplished through a pair of calomel half-cells held rigidly, at a fixed separation, in selected positions along the preparation. While the stimulating voltage is relatively independent of the nerve circuit, no attempt has been made to control the effect of differences in nerve resistance as the position of the electrode pair is changed. In many of the experiments the plan followed has been to obtain readings from one point on each of the roots at corresponding positions well away from the killed ends, and, from the trunk, at a number of positions including the region of the ganglion. Often in such experiments the location of the stimulating cathode was read with reference to the center of the ganglion. These values have been included in table 1.

Threshold readings depend to some extent on the amount of amplification employed. They may be decidedly lower when the amplification is sufficient to bring out single axon spikes than with the amplification reduced so that the full multifiber action potential is framed by the screen

TABLE 1*

NUMBER	LOCUS		RHEOBASE		REPETITION THRESHOLD				REMARKS
		Dist. from Gn.	M	S	M	S	M	S	
		cm.			*relative voltage*		*rheobases*		
1	Gn.		240	240	1000+	480	4.17+	2.00	Stimulation across Gn.
	Gn.		150	174	630	215	4.20	1.24	Cathode on Gn., anode on Tr.
	Tr.		51	55	400	102	7.53	1.85	
2	Tr.		187	200	260	225	1.37	1.12	
	Rt.		67	70	145	150	2.16	2.14	
3	Rt.		65	230	480	365	7.40	1.59	S rep. much better than M
4	Tr.								Distal. No rep.
	Tr.								Middle. S repeats, not M
	Gn.		380	450	1000+	600		1.33	
	Rt.		165	160	690	400	4.18	2.50	
5	Tr.		580	515	860	650	1.48	1.26	Distal
	Tr.		570	460	1000+	930	1.75+	2.02	Middle
	Gn.		270	480	1000+	750	3.70+	1.56	
	Rt.		130	110	590	300	4.53	2.73	
6	Tr.		620	410	800	720	1.29	1.76	Distal
	Tr.		560	460	1000+	960	1.79+	2.09	Middle
	Gn.		375	460	1000+		2.67+		S rep. poor
	Rt.		130	135	1000+	380	7.70+	2.81	
7	Tr.		54	50	175	90	3.24	1.80	Middle
	Tr.		62	55	190	100	3.07	1.82	Distal
	Gn.		50	86	250	114	5.04	1.33	
	Gn.		50	86	205	104	4.10	1.21	
	Rt.		100	138	255	410	2.55	2.97	Better S rep., large fibers. M small fibers
8	Tr.	0.30	73	82	1000+	245	13.70+	2.99	
	Tr.	0.75	80	73	1000+	245	12.50+	3.27	
	Tr.	1.95	58	54	1000+	1000+	17.25+	18.50+	
	Tr.	2.55	50	55	1000+	1000+	20.00+	18.20+	
9	Rt.		26	23	1000+	58	38.50+	2.52	
	Tr.	0.43	70	65	1000+	155	14.30+	2.38	
	Tr.	1.09	105	73	1000+	148	9.54+	2.03	
	Tr.	1.86	80	44	1000+	190	12.50+	4.32	
	Tr.	2.46	66	63	1000+	238	15.15+	3.78	

* The preparations are numbered consecutively in chronological order. The abbreviations Gn., Rt., Tr., Sc., M., S. and rep. signify ganglion, root, trunk, sciatic, motor, sensory and repetition. The values in a line and those in a brace are readings made with a setting of the potential divider; they are comparable among themselves. The figure 1000+ signifies that it was not possible with the particular voltage supplied the potential divider, to attain the voltage required to stimulate. Values derived from 1000+ are too small by an unknown amount, and this is indicated by a plus sign.

TABLE 1– *Concluded*

NUMBER	LOCUS		RHEOBASE		REPETITION THRESHOLD				REMARKS
		Dist. from Gn.	M	S	M	S	M	S	
		cm.		*relative voltage*			*rheobases*		
10	Tr.	0.67	48	50	187	157	3.90	3.11	
	Tr.	1.33	61	53	236	118	3.87	2.23	
	Tr.	2.44	72	62	160	270	2.22	4.35	
	Tr.	3.36	97	86	1000+	1000+	10.30+	11.62+	
11	Tr.		43	42	94	58	2.19	1.38	Good S rep., little M
12	Tr.		72	50	90	78	1.25	1.56	S rep. much better than M
				51		72		1.41	
13	Tr.		190	160	290	212	1.53	1.37	M rep. slight, S good
14	Tr.		77	60	115	85	1.49	1.42	M rep. poor
	Rt.		49	56	220	149	4.49	2.66	Low ampl.
					170	120	4.37	2.14	High ampl.
	Rt.		78	72	120	120	1.54	1.67	At 200 S rep. better than M
	Tr.		173	137	260	183	1.50	1.34	
15	Sc.		335	235	430		1.29	1.80	1st fiber. Max. S rep. slightly better than M
	Sc.		385	305	418		1.12	1.37	2nd fiber. Max. S rep. slightly better than M
	Sc.		323	272	438	412	1.36	1.52	1st fiber another locus. Max. S rep. slightly better than M
	Tr.		302	267	392	447	1.30	1.67	
16	Rt.		200	200	1000+	350	5.00+	1.75	Low ampl.
	Rt.		175	195	325	294	1.86	1.51	High ampl.
	Tr.		200	121	317	175	1.58	1.45	Max. rep. much greater in S
17	Tr.		242	143	790	304	3.26	2.13	High ampl. At rep. thresh. M fibers small, S largest
18	Tr.								S repeats, not M
	Rt.				900	610			S repeats slightly, not M
19	Tr.		200	155	435	240	2.17	1.55	S rep. good, M slight
20	Tr.			175		450		2.57	Sheath of trunk open
	Rt.			190		1000+		5.26+	
21	Rt.								High ampl., no rep.
	Tr.		97	164	1000+	200	10.30+	1.22	High ampl., S repeats
22	Tr.		225	200	400	236	1.78	1.18	High ampl.
				204					
23	Tr.		352	286	672	450	1.91	1.57	Most irritable fiber
24	Tr.		104	92		104		1.13	Most irritable S fiber repeats
25	Tr.		112	133					S rep. better than M
26	Tr.		121	105	290	150			Small M fibers, rep. poor, large S fibers rep. splendid
27	Tr.		173	150	295	262	1.70	1.75	Small M fibers and large S fibers rep., both poorly

of the electron oscillograph. Usually the relative excitabilities of the motor and sensory fibers have been the same at the different amplifications. Occasionally, however, there have been wide differences due to the presence of one fiber, or of a few fibers, of outstanding excitability whose spikes do not contribute appreciably to the picture produced at the lower amplification. An illustrative set of observations will be found under 16 in table 1. There with the low amplification the motor and sensory rheobases were both 200;[2] with high amplification they were, respectively, 175 and 195: increasing the amplification lowered the reading of the motor rheobase 12.5 per cent, that of the sensory rheobase only 2.5 per cent, and this is by no means the widest shift that has been seen.

The gross differences that have been observed between the rheobases in the different positions along a preparation are attributable largely to gross anatomical features. The voltages required to stimulate *the roots* always are relatively low (see table 1) due, probably wholly, to the absence from the roots of an epineurial covering. The average motor rheobase in twelve preparations was 117, the sensory rheobase 134. In six instances the motor rheobases were the higher and in one instance the two rheobases were essentially the same. The distribution around the mode, therefore, is nearly even. Included in this group, however, is one case (3, table 1) in which the sensory rheobase was greatly in excess of the motor rheobase. This was so exceptionally large that for statistical purposes it seems justifiable to exclude it. When this is done the averages become almost alike, —121 for the motor fibers and 125 for the sensory. However, the root thresholds, as measured, are, as has been said, qualified to an unknown extent by possible differences in the relative sizes of the roots and in the contacts between electrodes and roots.

On the trunk just beyond the ganglion the motor threshold usually is the lower (see fig. 1 and table 1), probably because here the motor fibers are the more accessible to the current. This difference becomes even more striking when the motor and sensory thresholds are determined with the cathode on the ganglion. The motor and sensory thresholds in these particular regions are of no value from the comparative standpoint. Further along on the trunk where the two kinds of fibers presumably are uniformly intermingled and ensheathed in the epineurium all of these conditions qualifying comparison should disappear. There the thresholds, both motor and sensory, are generally high relative to those of the root, and the motor threshold is higher than the sensory. To be specific, the motor rheobase has been higher than the sensory in thirty of thirty-six comparisons. The averages of all of the motor and sensory rheobases are to each other as 120 is to 100, and this difference is, if anything, too small

[2] Throughout this paper rheobases are expressed in terms of relative voltage, and only those values are comparable that apply to the immediate context (see footnote to table 1).

since any determinations derived perchance from the regions contiguous to the ganglion, where the threshold curves are crossing (see fig. 1), would raise the average of the sensory rheobase above its correct value.

Curiously enough the ratio of the motor to the sensory rheobase, as may be seen in the table, is by no means the same either quantitatively or even directionally at different, though presumably comparable, points on one and the same trunk. No reasonable explanation of these differences has occurred to us. Granting even that there are local demarcation currents which modify excitability locally it is difficult to see how they could affect motor and sensory fibers differently unless the two kinds of fibers react differently to them or unless the motor and sensory fibers course in separate bundles which are unequally exposed to such demarcation currents and to the stimulating currents.

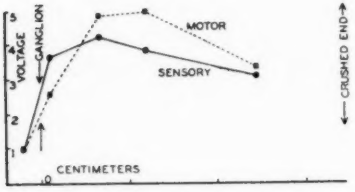


Fig. 1

Fig. 2

Fig. 1. Rheobasic voltage (arbitrary units) along a root-trunk preparation. Zero of distance is the center of the ganglion. The roots are to the left, the trunk to the right.

Fig. 2. Records of multifiber spikes showing the comparative repetitiousness of motor (upper) and sensory (lower) fibers. The motor spike exceeds in height the limits of the screen and, because of high vertical speed, it records only close to the base line. Sensory *alpha*, even when maximum, *F*, never exceeds screen limits. Amplification same for all. Time = 1 kilocycle. Unretouched. Natural size.

As a measure of the *comparative repetitiousness of motor and sensory fibers* or of any one kind of fiber at different localities, the threshold of repetition is valid only when the rheobases are alike. On the other hand the repetition threshold in terms of rheobases, the "relative repetition threshold", should be a valid comparative measure of repetitiousness under any conditions. However, under conditions apparently quite comparable, the number and the sizes of the fibers (as judged by axon spike height) that can be made to repeat has varied very widely not only with the preparation but also from point to point in the same preparation, an experience which confirms observations on the plantar preparation (Erlanger and Blair, 1936). Consequently the information yielded by either of the above-mentioned gauges of repetitiousness requires statistical treatment. As a third measure of repetitiousness there is the maximum amount of repeti-

tion, as given by area, elicitable under comparable conditions. This method is regarded as entirely satisfactory when due consideration is taken of the relative fiber constitution of the two contributions. It has not been necessary actually to measure the areas, since the differences ordinarily have been so great that inspection of the tracings on the screen, or of the photographs, has sufficed for a decision (see fig. 2). It must be borne in mind here that the repetition induced by a constant current does not increase to a constant value with the current strength; instead it passes through a maximum which is attained usually at from 3 to 4 rheobases (Erlanger and Blair, 1936). To supplement the data in table 1 it should be added that the repetition maximum yielded by the sensory fibers has been so regularly larger than that yielded by the motor, that it has been convenient to include under "Remarks" only notes on unusual results, such as equality of motor and sensory repetition, unusually small or unusually large differences between the motor and sensory repetition maxima, *et cetera*.

Table 1 includes eleven comparable determinations of the *repetition threshold in the roots*. In seven of these cases it was lower in the sensory root than in the motor; in two the roots had the same threshold; in one case neither root repeated; and in one the repetition threshold was higher in the sensory root.

Data on the *relative repetition threshold* (the repetition threshold in terms of the rheobase) *in the roots* are found in eight experiments. In five this value is higher in the motor root than in the sensory; in one case it is the same for both roots; and in two (nos. 7 and 14) the motor root ratio is smaller than that of the sensory, though in one of these only slightly so.

The repetition maximum, where repetition has been attainable at all, has been greater in the sensory root than in the motor in every instance save one, namely, experiment 7 of table 1, in which the note was made that the repetitiousness of the two kinds of fibers was about the same. This is the one case, referred to in the previous paragraph, in which the repetition threshold was decidedly higher for the sensory than for the motor fibers. In the other instance with this relation of these thresholds (14, table 1) it was noted that with stronger currents the sensory fibers repeated *better* than the motor. Statistically, therefore, the observations signify definitely that the sensory roots contain fibers that repeat more readily than any of the fibers in the motor roots. It should be added, though, that the difference in the motor and sensory maximum of repetition has never been as great in the roots as in the trunk.

Repetition thresholds in the trunk can be of significance from a comparative standpoint only when derived from regions that are uniformly constituted. Therefore, of the data contained in table 1, only such will be considered

under this rubric as are known, or are believed, to have been derived from points 5 mm. or more from the ganglion. Thirty-one pairs of observations on eighteen preparations meet this condition. The strongest current needed to elicit repetition, where repetition was elicitable at all, was, with one exception (and this was 7.5 rheobases), in the vicinity of 4 rheobases. Therefore, where the drop across the potential divider sufficed to permit of the quadrupling of the rheobase and that increase failed to elicit repetition, it is assumed, somewhat arbitrarily, to be sure, that the fibers would not have repeated with any strength. This obtained eight times with motor fibers and three times with sensory. All three of the latter were in preparations of which neither the motor nor the sensory fibers could be made to repeat.

It has been possible to compare repetition thresholds of the motor and sensory fibers in twenty-nine of the locations on the trunk. In these the sensory repetition threshold was lower than the motor in twenty-seven instances. This series includes five preparations of which the most irritable motor fiber and the most irritable sensory fiber were under observation. It may be of interest to note that of the two exceptions to the rule just mentioned one was in this single fiber group.

Since there is the possibility that even in the trunk the two kinds of fibers may not be equally exposed to the stimulating current, a safer comparison is one based on the current needed to stimulate in terms of the rheobase. Information required for this comparison has been derived from twenty-six loci. In nineteen the sensory threshold thus expressed was the smaller of the two. The series includes all five of the single fiber observations mentioned above; and of these the sensory threshold was the smaller in only two. This last result is probably an expression of the fact, mentioned above, that repetitiousness is in part a matter of fiber individuality. Therefore, of the two methods, the one that is based on the behavior of a number of fibers should be the better index to comparative repetitiousness.

As in the case of the roots so in the trunk, if repetition was elicitable at all, the area of the maximum of the repetition obtainable from the sensory fibers has been either greater than that from motor fibers or, in the few instances in which this did not obtain, the two areas have been approximately alike. Figure 2 illustrates a typical case. The rheobase, the repetition threshold and the relative repetition threshold of the motor fibers here were, respectively, 190, 290 and 1.53, of the sensory fibers, 160, 212 and 1.37. Records *A*, *B* and *C* are the responses obtained at, respectively, a subrepetition strength of the constant current (at 260), at the repetition threshold (290) and at about the repetition maximum (700) of the motor fibers; and records *D*, *E* and *F* are the responses at corresponding situations for the sensory fibers, *D* at a strength of 200, *E* at 212 and *F* at 650.

The question arises here, *is the difference in the motor and sensory repetitiousness referable to differences in the sizes of motor and sensory fibers?* Is there among the sensory fibers a predominating size more prone to repeat than any other? In the roots the largest medullated fibers of both kinds are essentially alike in size and so too are the smallest (Gasser and Erlanger, 1927). In the different roots, however, there may be considerable variations in the frequency distribution of the fiber sizes (Taira, 1934, 1935), and there are no reasons for believing that this relation is greatly different in the trunk. The greater repetitiousness of the sensory fibers cannot be attributed to a predominance of small fibers, since it is easy to show that at the repetition threshold of the sensory fibers, it is the large fibers (judging by spike height) that are repeating. Indeed, in more than one instance (though by no means regularly) it has been found that the height of the spike of the sensory fiber repeating at the repetition threshold was the same as that of the fiber with the lowest rheobase: the largest fiber was repeating. Moreover, it has happened in a preparation in which the large sensory fibers were repeating, that at the very much higher repetition threshold of the motor fibers smaller motor fibers were the ones that were repeating.

The experiment and records (fig. 2) described above can be used to demonstrate that the greater repetitiousness of the sensory fibers is not attributable to a preponderance of small fibers on the sensory side. The repetition, both motor and sensory, begins there long before the *alpha* spikes attain their maximum heights and before, on the sensory side, the stimulus reaches the *beta* threshold. To be specific, the first evidence of motor repetition appears when *alpha* has attained 75 per cent of its maximum amplitude; and the first evidence of sensory repetition develops as the sensory *alpha* attains about 36 per cent of its height.

This question of fiber size in relation to repetitiousness has been gone into rather thoroughly and, without presenting any more of the evidence that has been accumulated, it may be stated that we have satisfied ourselves that a characteristic difference in the sizes of sensory and motor fibers is not the factor that is responsible for the difference in motor and sensory repetitiousness.

Comparative repetitiousness of root and trunk fibers. The fibers of the roots repeat much less readily and much less perfectly than do those of the trunk. In six pertinent observations on motor fibers the relative repetition threshold of the root fibers was higher than that of the trunk fibers in five; in one instance the situation was reversed. In eight pertinent observations on sensory fibers the rule held in seven; in the remaining case the figures from several points on the trunk ranged around the one derived from the root. Moreover, for any one set of fibers the repetition maximum, when repetition was obtainable, has without exception been greater in the trunk than in the root.

This difference between root and trunk recalls observations on the comparative repetitiousness of the sheathed and the desheathed phrenic nerve; —the fibers in the former are much more apt to repeat than are those in the desheathed nerve (Adrian, 1930; Erlanger and Blair, 1936). With a view to ascertaining whether the sheath is a factor in the present case the following experiment has been performed. A preparation was selected in which the motor root joined the trunk just beyond the ganglion, as it occasionally does. While drawing the roots apart, the separation was extended with scissors 3.5 mm. along the trunk beyond the edge of the ganglion. A pair of electrodes, for constant current stimulation, was applied with a terminal 3 mm. to either side of the ganglion. When the cathode was on the trunk side the record was taken from the trunk, and when it was on the sensory root the record was taken from that root. The rheobases of the central and peripheral extensions from the sensory ganglion determined in this way were, respectively, 190 and 175 (table 1, 20). The repetition threshold of the peripheral extension (the trunk fibers) was 450, while the central extension (the root fibers) could not be made to repeat. The corresponding relative repetition thresholds were 2.57 and 5.26+. The result is the one given by normal preparations. Opening the sheath of the trunk, then, does not alter the repetitiousness of the sensory fibers. The experiment, however, may not be crucial since the operation does not completely remove the sheath, and undoubtedly damages some of the fibers.

Accommodation. Since, under a constant current, sensory fibers repeat more readily than motor fibers it is to be expected that during the passage of a subthreshold constant current sensory fibers accommodate less than do motor fibers. This is the case.

During the passage of a constant current, as is well known, the threshold at the cathode falls to a level which usually is ended abruptly, after about 1 to 2 msec., by a rise in threshold (accommodation) which approaches asymptotically the steady elevated value that is maintained by the continuing polarization (Erlanger and Blair, 1931, 1936). The amount of accommodation is expressed by the ratio of the threshold obtaining when the rise during polarization attains its asymptote, to the threshold of the unpolarized fibers. When a comparison is to be made between the grades of accommodation developed in different preparations or in different fiber groups of the same preparation, the polarizing currents employed should be proportional to the respective rheobases, and this precaution has been observed. The polarizing current employed in the present experiments has been 80 per cent of the rheobase and the shocks determining the amount of accommodation have been administered 10 msec. after the make of the constant current.

The results obtained from two preparations are given in table 2 along with other data. There it can be seen that in both cases the accommoda-

tion ratios are smaller for the sensory than for the motor fibers. The data on the repetitiousness of the fibers of these preparations will be found in table 1 as numbers 26 and 27: as usual, the sensory fibers were more repetitious than the motor.

Demarcation potentials. In the effort to find differences between motor and sensory fibers which might correlate with the difference in their repetitiousness, their demarcation potentials have been measured. This has been done under exactly comparable conditions. The apparatus employed has been a Leeds and Northrup potentiometer, type K, in conjunction with a type HS Leeds and Northrup galvanometer with sensitivity of approximately 1×10^{-10} ampere, permitting readings easily within 0.05 mv. The electrodes were calomel half-cells. The potential difference between

TABLE 2

	EXPERIMENT 26		EXPERIMENT 27	
	M	S	M	S
Rheobase, *relative voltage*	972	865	1000	885
Shock excitability, *primary ohms*	1150	1205	1720	1720
Shock threshold, *relative voltage*	86.9	82.8+	58.2	58.2
$\frac{\text{Rheobase}}{\text{Shock threshold}}$	11.18	10.45	17.18	15.20
Shock excitability in plateau, *primary ohms*	2180	2310	3300	3300
Shock threshold in plateau, *relative voltage*	45.9	43.3	30.3	30.3
Shock excitability, polarized* 10 msec., *primary ohms*	1682	1900	2420	2650
Shock threshold, polarized 10 msec., *relative voltage*	59.5	52.6	41.3	37.7
Accommodation ratio $\left(\frac{\text{Thres. polarized}}{\text{Thres. unpolarized}}\right)$	0.684	0.634	0.711	0.648

* The polarizing current was 80 per cent of the threshold.

the electrode pairs, never exceeding 1 mv., was steady and was deducted from the readings. Killing was accomplished by heating with either a tetanizing current or Ringer's solution. The results obtained were not perceptibly influenced by the method of killing employed. In one set of experiments the central ends of the roots were killed and the leads were from one or the other killed end and from the intact trunk distal to the ganglion. In another set (see fig. 3, B) the common lead was from the union of the two roots, which was killed together with the ganglion, while the other two leads were one from each of the roots about midway between their cut ends and the killed region in the vicinity of the ganglion. The latter series included readings of the potential difference between the two roots. It should be added that the potential difference between the roots

before killing was insignificant. All the readings were made without moving the electrodes.

The results were all qualitatively alike (see fig. 3, A). The motor root demarcation potential invariably is higher than that of the sensory root. In the eight preparations examined the difference immediately after killing ranged between 3 and 13 mv., with an average of 6.3. This wide spread of the difference between the motor and sensory demarcation potentials in different preparations is not referable to differences in the thoroughness of the killing, since both methods gave equally wide ranges; and when the trunk ends of the roots are killed the sensory and motor fibers obviously receive similar treatment. Neither does it correlate with root diameters. The potential difference between the two electrodes resting on intact

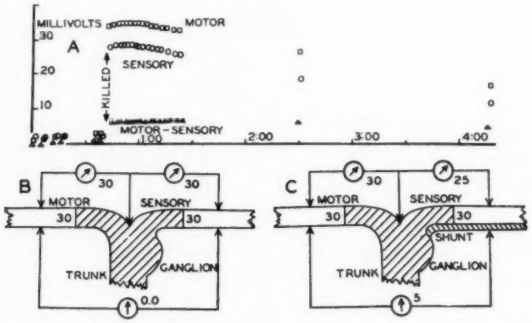


Fig. 3. A. Motor (squares) and sensory (circles) demarcation potentials. Triangles: motor to sensory demarcation potentials. Time in hours. B and C. Diagrams illustrating arrangement of leads, and a possible explanation of the results obtained. Killed region is cross-hatched.

surfaces, one on the motor root, the other on the sensory, was always equal to the difference between the two demarcation potentials (see fig. 3, A).

Diagrams (fig. 3, B and C) will help to visualize the results obtained with the arrangement in which the union of the roots was killed. Starting with the premise that the potential drop between the intact surface of either root and the killed root junction is 30 mv., as indicated in diagram B, the drop between the two roots obviously would be zero. If, however, as indicated in C, there were a 25 mv. drop between sensory root and killed region, a 30 mv. drop between motor root and killed region and a 5 mv. drop from the sensory to the motor root, the situation might be referable either to a potential difference inherent in the roots or to inert shunting material greater in amount in the sensory than in the motor root, as shown in C. Now if the latter were the case, while all other relevant conditions in the two roots were the same, the constant current threshold of the sensory

root would be higher than that of the motor root, but as has been seen, there is little if any difference between the roots in this respect; and in the trunk, if trunk values are relevant here, the sensory rheobase is very much lower than the motor. It would appear, therefore, that the results obtained are referable only to an inherent difference between the demarcation potentials of the motor and sensory fibers.

Such a difference in demarcation potentials might be related to the greater repetitiousness of the sensory fibers either as effect or as cause. Thus the potential difference between the two roots would be accounted for if the sensory fibers were firing off spontaneously and the motor fibers not. There is, however, no spontaneous activity to be seen in either kind of fiber under resting conditions. On the other hand, if the difference in demarcation potentials were an expression of a difference, say, in the thickness of a hypothetical membrane, that of sensory fibers being the thinner, it is obvious that under the influence of a constant current the sensory fibers would be more repetitious than the motor.

The temporal configurations of the curves of the demarcation potentials vary somewhat from preparation to preparation. Generally the motor injury potential, starting at, say, 30 mv. increases for about 15 minutes to reach, say, 33 to 35 mv. before beginning to decline. Occasionally, however, it has fallen from the start, and then this initial fall has been slower than the contemporaneous fall occurring in the sensory root. The sensory demarcation potential may also rise initially but then the rise is relatively slight. Usually, however, the sensory demarcation potential falls from the start. Eventually, it may be after two hours or longer, the two curves, while falling, begin to approach each other. One preparation has been followed through 17 hours. At the end of that period the sensory injury potential was 0, the motor 3.5 mv. The initial difference had been 6 mv.

Strength-duration relationships. Another difference between motor and sensory fibers that has been encountered concerns their thresholds to currents of long and of short duration. The phenomenon came to light in an experiment in which the rheobasic voltages of the most irritable motor fiber and the most irritable sensory fiber of a preparation had been determined, and then their excitabilities in terms of threshold induction shocks. The stimulating electrodes were on the trunk and the responses were being led separately from the motor and sensory roots. The rheobase of the sensory fiber was lower than that of the motor, but the shock threshold of the sensory fiber was the higher of the two. In another instance (table 1, 23) the reciprocals of the primary resistances (the relative primary voltages) when the shocks were threshold for the most irritable motor and most irritable sensory fibers, were, respectively, 8.00 and 7.94; essentially they were the same. The rheobases of these two fibers were, respectively, 352 and 286: the sensory fiber, by the latter test, was 23.1 per cent more

irritable than the motor. Two additional instances are shown in table 2, where it is seen in each case that the ratio of rheobase to shock threshold is larger for the motor fiber than for the sensory fiber. In figure 4 are reproduced complete strength-duration curves derived from two fibers in another preparation. The motor and sensory curves here actually cross, the threshold of the sensory fiber being the lower at long durations and the higher at short durations. The shift, to be sure, is small, but it was readily demonstrable. The sensory chronaxie here is 0.19 msec., the motor 0.16.

In this connection it may be recalled that strength-duration curves of *sensory* fibers of different velocities differ in configuration in such a manner that as the conduction rates of the fibers slow their chronaxies at first

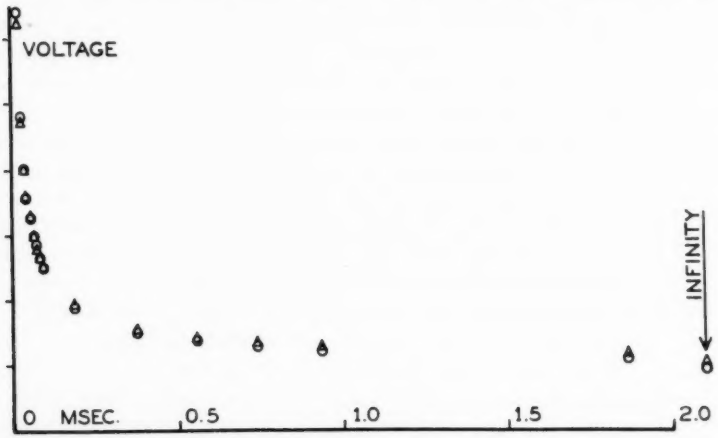


Fig. 4. Strength-duration curves of the most irritable motor fiber (triangles) and the most irritable sensory fiber (circles) of a preparation.

shorten and then lengthen (Blair and Erlanger, 1933). In that investigation we did not encounter crossing of curves, though we did not "expressly say that crossing of fiber curves is not seen."[3] Crossing of strength-duration curves has, however, been described by others, most recently by Schaefer (1936,—where the literature will be found), who maintains that diminution in chronaxie with fiber size and crossing of curves are necessary consequences of the local potential (*Spannungs*) theory of electrical stimulation of nerve. Schaefer, however, used constant potential height of multifiber responses as the criterion of constant stimulation strength, and this method is not wholly valid since the briefer the threshold current the less is the temporal dispersion of the axon spikes elicited, and, therefore,

[3] The quotation is a translation from Schaefer (1936), who imputes this statement to us.

the more complete their summation. Under the circumstances, however, the question as to whether there is a characteristic difference between the strength-duration curves of motor and sensory fibers must remain open. Nevertheless, the results described here, it will be shown, are not inconsistent with the hypothesis ascribing to the sensory fibers the thinner "membrane".

Conduction rate; multifiber observations. Since of the largest fibers of the trunk the sensory are definitely more excitable than the motor, one might expect conduction in sensory fibers to be faster than in motor fibers. As a matter of fact the reverse appears to be the case, and this difference presumably constitutes another of the significant differences between motor and sensory fibers.

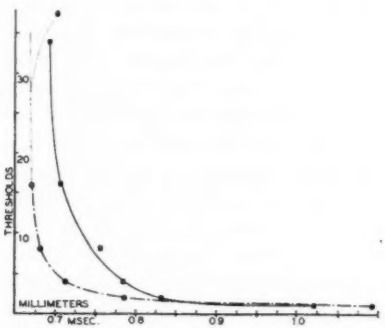


Fig. 5. Effect of stimulus strength on shock-spike time including time for conduction. The distance between stimulating electrode and lead is fixed in each set. The lead is from the trunk. Motor root stimulated, circles; trunk stimulated, squares. The upper scale on the abscissa gives distance covered, assuming a velocity of 40 M per sec.

Conduction has been studied in twenty-eight different preparations. In all cases determinations have been made of the time occupied by propagation between successive, rather closely spaced, points. Two general methods of determining the conduction time have been used. 1. In some cases the roots were stimulated separately at fixed points with induction shocks and records were taken from the ground lead which was moved to the selected points along the roots and the trunk, the grid lead remaining fixed on the killed end of the trunk. 2. In other cases two pairs of leads which could be used separately, were in fixed positions one on each of the roots, and the points stimulated were selected by shifting the pair of stimulating electrodes.

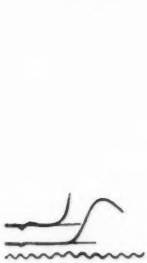
After preliminary experiments with methods the stimulus strength usually employed was exactly twice threshold in each position. In two

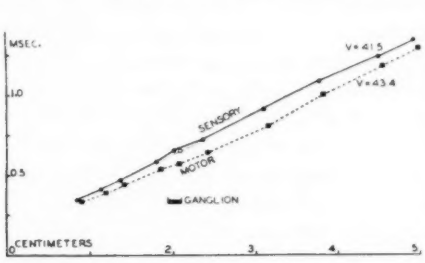
cases the strength was adjusted to six times threshold (see fig. 9). The need for exercising this precaution was ascertained in experiments in which the effect was determined on the shock-spike time (with conduction) of increasing the strength of the shock. A pair of curves illustrating the results obtained, one when the shock was applied to the trunk and the other when it was applied to a motor root, are shown in figure 5. There it is seen that increasing the strength to about sixteen thresholds in the trunk and to about eight thresholds in the root shortens the time 0.31 and 0.41 msec., respectively, a spread that is equal to the time required for the impulse to travel a distance that is about as long as is available for observation in roots. With further increase in stimulation strength the shock-spike time may actually lengthen (see root curve, fig. 5). This lengthening could be due only to an effect of an escape of stimulating current along the preparation, which modifies conduction of the after-coming action potential. Stabilization of the shock-spike time then could be attained by using shocks eight to sixteen times the threshold strength. Such strong shocks, however, eventually damage some of the fibers. For these reasons a compromise was adopted and, as stated above the shocks, in most cases, were carefully adjusted to twice threshold. Amplification then was adjusted to keep the resulting action potentials all at the same recorded height. Time was recorded, usually, in the form of 10 to 25 kilocycle oscillations.

With the high speed of deflection needed in these experiments it is rarely possible to pick with sufficient accuracy the point at which the recorded action potential rises out of the base line,—a difficulty that usually is enhanced by distortion of the base line by the shock artifact and by base line artifacts that develop whenever the traveling action potential traverses a locus of change in diameter of the preparation (Bishop, Erlanger, and Gasser, 1926). The significance of this difficulty is made evident in figure 6. These are records of the same multifiber action potential with different amplifications, the amplification factors of the lower and upper records being to each other as 1 is to 20. The records show that at low amplification the actual start of the spike cannot be located. In order to minimize error from this source the conduction time, the shock-spike time, was measured to points on the rising limbs of the spikes at an adequate percentage (between 10 and 20) above the base line. The conduction time ascertained in this way is affected by the change in the configuration of the spike with conduction. One would expect the rate of rise, of the sensory spike particularly, to decrease with the distance. Curiously enough, however, the slopes, within the distances conduction has been followed, often have changed insignificantly. In one experiment the time was measured both to the apparent foot and to a point 10 per cent of the rising phase above the foot. The conduction rates thus determined turned out

to be faster (but almost within the limit of error) with the latter method. All measurements have been made after about eight-fold enlargement of the photographs.

Conduction rate graphs, which may be regarded as more or less typical of those obtained through movement *a*, of the stimulating electrodes, and *b*, of the recording ground electrode, are reproduced in figures 7 and 8, respectively. In figure 7 the conduction rate in the roots, as shown by the slopes of the curves, is slower in the sensory than in the motor fibers; in the trunk and sciatic nerve the motor conduction rate is the slower. The differences, however, are not great, and the conduction rate through





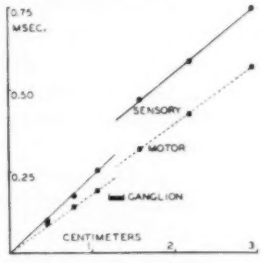


Fig. 6 Fig. 7 Fig. 8

Fig. 6. Effect of amplification on the measurement of shock-spike time. The conditions for the two records are identical excepting that the relative amplifications are as 1 (lower) is to 20 (upper). The records are placed with their shock artifacts on the same vertical. Time = 7 kilocycles. Natural size.

Fig. 7. Graph of conduction time against distance, multifiber records; motor (squares) and sensory (dots). Lead fixed, stimulus moved. Time and distance are with reference to shock and lead electrode, respectively. The roots are to the left, the trunk to the right, of the "ganglion".

Fig. 8. Graph of conduction time against distance, multifiber records; motor (squares) and sensory (dots). Stimulus fixed, lead moved. Zero time here is the time of arrival of the spikes, motor and sensory, at the most proximal lead point. Otherwise as in figure 7.

the entire length is somewhat slower in the sensory fibers (41.5 m.p.s.) than in the motor fibers (43.4 m.p.s.). The major irregularities in the curves are in the vicinity of the ganglion and probably are referable to the effect the ganglion and the sheath exert on the spread of current lines from the stimulating electrode. The dot on the part of the sensory graph that is over the ganglion is the reading obtained when the stimulating cathode was on the ganglion and the anode on the trunk. It is more than likely that stimulation under these circumstances occurred at the edge of the ganglion. The open circle marks this position. When the graph is made to pass through the circle (dotted line), instead of through the corresponding dot, this part becomes essentially linear.

In the case of figure 8 the conduction rate is slower in the sensory than in the motor fibers, both in the roots and in the trunk. The sensory graph, however, shows a discontinuity in the region of the ganglion which might be interpreted as a lag of the action potential there amounting to about 0.14 msec. The motor curve shows a discontinuity there also; it is in the same direction as the sensory discontinuity but much less significant. This discontinuity in the graph of transmission of the sensory impulse through the ganglion was described by Erlanger, Bishop and Gasser in 1926, using a method of following conduction rates comparable with the one supplying the data for figure 8, excepting that the shock-spike times were taken as the conduction times. It was regarded by them as evidence of a lag in the transmission of the sensory impulse through the ganglion. Since, however, we do not now find a lag regularly when all of the precautions are observed that were employed in obtaining the data for figure 7 it seems necessary to conclude that the lag is an artifact. Speaking now for ourselves only, we are inclined to attribute the apparent lag to an effect the sheath exerts on the transmission of the intrinsic potentials to the recording grounded electrode. The sheath must have the effect of broadening the lead and of delaying, through this effect, the rise of potential as the active locus leaves the ground. But why this affects the sensory action potential more than the motor is not now clear.

The present experiments were performed primarily for the purpose of obtaining data for a comparison of sensory and motor conduction rates. Twenty-three preparations supply the necessary information relative to the roots. The sensory conduction rate was slower than the motor in thirteen of these cases, faster in five and the motor and sensory rates were the same in five. Of twenty-four observations on the trunk the sensory conduction rate was slower than the motor in twelve, faster in eight and the same in four. In other words, it is rather the rule for the fastest sensory fibers to conduct more slowly than the fastest motor fibers. This obtains despite the fact that in the trunk the excitability of the sensory fibers is, as has been seen, almost invariably higher than that of the motor fibers.

Conduction in single fibers. Attempts to follow conduction through single motor and single sensory fibers have led to relatively little of interest as regards the principal thesis, mainly owing to lack of assurance that the same fiber has been stimulated along the length of the preparation. In several cases there has been encountered what has appeared, through the all-or-none responses, to be a fiber with an outstanding excitability everywhere. When, in such instances, the derived graphs of conduction along the preparation have been linear one can be reasonably certain that a single fiber has been traced. Two such sets of observations are available. In figure 9 the spike of a motor axon is followed through root, trunk and sciatic

nerve. Conduction through the latter two regions is linear and the bend in the curve between root and trunk is not any greater than is frequently observed there in multifiber experiments. The spike of the most excitable sensory fiber also was elicited through the preparation; the graph, however, shows discontinuities, indicating, probably, that fibers were exchanged along the preparation. Strangely enough the conduction rate in the motor fiber in the trunk and sciatic was 20.3 M per sec., and in the sensory fiber 34.6 M per sec., whereas the shock thresholds were as 1.75 is to 2.5. In other words, the more excitable fiber has the lower conductivity. And stranger still is the observation that the motor multifiber conduction rate (34.6 m.p.s.) in the same preparation was very much faster than that of

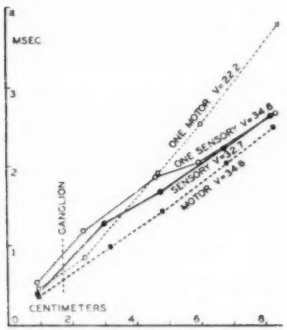


Fig. 9

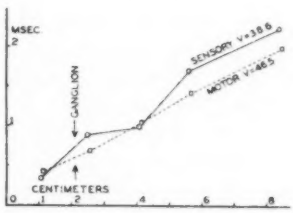


Fig. 10

Fig. 9. Graph of conduction time against distance. Squares, motor, single fiber; blocks, motor, multifiber, 6 × threshold; circles, sensory, single fiber; dots, sensory, multifiber, 6 × threshold. Zero of time and distance as in figure 7.

Fig. 10. Graph of conduction time against distance for the most irritable motor fiber and the (?) most irritable sensory fiber. Zero of time and distance as in figure 7.

the most irritable motor fiber, the one referred to above. On the sensory side, however, the single fiber and the multifiber conduction rates were essentially alike.

Another motor fiber apparently has been traced in figure 10, the curve being linear; in this case, however, the most excitable motor fiber has the reasonable conduction rate of 46.5 M. per sec. Here again, the complexity of the sensory graph indicates that the same sensory fiber has not always been stimulated at the several loci. If the irregularity of the curves is an expression of the intrusion of different fibers, the former must be interpreted as signifying that the most irritable fiber at one locus has a very different conduction rate than the most irritable fiber at another. It may be added, that there was no correlation here between the local velocities

of sensory conduction and the local excitabilities, unless it is assumed that two fibers were being traced, and then the data indicate that the more irritable of the fibers had the *slower* velocity and the *lower* spike. This interpretation does not appear to be unreasonable, in view of the great difference in the velocities in the most irritable motor fiber in each of the two cases discussed above.

Conduction failure through the ganglion. It has happened not infrequently during an experiment that, of the motor and sensory fibers, the sensory only have failed to conduct. This failure as a rule has developed more or less gradually; but in a few instances it has come on with startling rapidity. When, in such instances, the search has been made, the fault has been located in the ganglion. If, as those observations indicate, the ganglion is a point of least resistance, it seemed that this vulnerability should be demonstrable through exposure of the preparation to untoward conditions such, for example, as oxygen lack or overwork.

To observe the effect of *oxygen* lack the method employed was to expose the entire preparation first to an atmosphere of oxygen and then to an atmosphere of hydrogen, all the while stimulating the motor and sensory roots alternately and equally, and leading the responses to stimulation either from the region of the ganglion or from the trunk beyond the ganglion. The gases were thoroughly washed and saturated with water. The records gave no evidence of a consistent difference in the rate of failure of motor and sensory conduction.

To test the *capacity* of the motor and of the sensory fibers *to carry impulses* through the region of the ganglion two general methods of procedure have been followed. *a.* Either the trunk has been stimulated continuously at high rates while leading alternately from the sensory and motor roots, or *b*, while leading from the trunk, the roots have been similarly stimulated, but alternately, for equal periods of time and with currents equally strong relative to the respective thresholds. Stimulation was effected through an 885 tube and the rates employed rose to about 500 per second. Stimulation fatigue was controlled by shifting the locus stimulated.

As the rate of stimulation approaches the above rate the height of the spikes falls off, alternates and then becomes irregular, but the motor and sensory pictures derived from six different preparations on the average have changed alike in all respects through long periods of stimulation.

Leads from the ganglion. A comparison of records taken along the preparation in a variety of ways discloses no discontinuities in amplitude or duration of spikes which might be referred to the entrance of an increment of potential from the ganglionic region. Furthermore, analysis shows that the area of the sensory spike bears a constant relation to that of the motor spike (within 3 per cent) whether the lead be from the ganglion or from the trunk. Neither the cells nor the connecting

fibers give rise to any potential that can be recognized by the variety of methods employed.

DISCUSSION. As a working hypothesis to help with the experimental development of this problem it has been assumed, as has been seen, that the sensory fibers differ from the motor in having the looser membrane. All of the observations seem to fit into this view: sensory fibers have the lower threshold, accommodate less and therefore are more repetitious; they have the lower demarcation potential, and the greater relative responsiveness to currents of long duration as compared with brief currents. Has this hypothesis, then, an anatomical basis?

1. The peripheral sensory fiber is regarded generally as a dendrite, the motor and the sensory root fibers as axons. The experimental findings cannot, however, be made to fit this anatomical classification. Repetition, to be sure, reaches its highest development in the peripheral sensory fibers; but the central sensory repeat somewhat better than the motor fibers, both axons. Moreover, the difference in demarcation potentials recorded here applies to the root fibers; information as to how the motor and sensory fibers behave in this respect in the trunk seems unattainable, since there they do not lend themselves to discrete determinations.

2. If we are dealing with a difference in membrane tightness, what constitutes the membrane? Jasper and Monnier (1933) have assigned this rôle, at least in part, to the myelin sheath. At any rate they attribute the high repetitiousness of crustacean nerve to the absence of this sheath with its current limiting capacity. This view does not, however, appear to be applicable here, since we are not familiar with any evidence indicating that for fibers of the same large size the myelin sheath of sensory fibers is thinner than that of motor fibers.

Moreover, evidence from this laboratory indicates that electrical currents stimulate (and block) medullated nerve fibers, not through the medullary sheath, but through the nodes of Ranvier (Erlanger and Blair, 1934). From this standpoint it would seem that the main difference between medullated and nonmedullated fibers consists of the fact that one has insulated segments, the other not, and that in the case of medullated fibers differences in "membrane tightness" are largely a matter of the relation obtaining between nodes and internodes.

In the absence of any specific information on this subject it would profit little to attempt much more than an enumeration of the possibilities. As compared with motor fibers, in sensory fibers either the nodes might be wider, or the internodes shorter. This investigation includes one observation that can be accounted for through the latter alternative. It has been seen that in the trunk the sensory fibers are much more excitable than the motor, and yet conduct at about the rate of the motor fibers. If conduction is effected through restimulation by eddy currents and if it is saltatory,

the tendency the high excitability must exert toward hurrying propagation might be counterbalanced by the increased number of restimulations needed per unit of distance traversed.

SUMMARY

On the basis of evidence indicating that the sizes of the largest sensory and motor fibers in the bullfrog are essentially alike this investigation shows:

1. That the rheobase of peripherally directed sensory fibers is at least 20 per cent below that of motor fibers.

2. That the repetitiousness of sensory fibers exceeds that of motor fibers as measured by:

a. The constant current threshold of repetition,

b. The constant current threshold of repetition in terms of rheobases, and,

c. The maximum amount of repetition elicitable by a constant current.

3. That during the passage of a constant current sensory fibers accommodate less than motor.

4. That the peripherally directed sensory fibers are more repetitious than the centrally directed, and that this difference probably is not assignable to the difference in the ensheathment of root and trunk.

5. That the demarcation potential of the sensory root is lower, by roughly a third, than that of the motor root.

6. That sensory fibers as compared with motor are more responsive to currents of long duration and less responsive to currents of short duration.

7. That despite their greater responsiveness to currents of long duration sensory fibers conduct perhaps less rapidly than motor fibers.

These results can be fitted into the hypothesis that sensory fibers have the "looser membrane"; and reasons are given for locating the "membrane" at nodes rather than at internodes.

Occasional rather sudden failure of the sensory fibers to conduct through the ganglion has been observed; yet conduction through the ganglion withstands oxygen want and a high rate of response as well as does conduction through motor fibers.

With improved methods it has not been possible to substantiate a lag in the propagation of the impulse through the ganglion. Neither has it been possible to find any evidence for a diversion of action potentials traveling in sensory fibers into their cells.

An exceptional relation is described between conduction rate and excitability in a motor fiber.

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A THEORY OF RETINO-CEREBRAL FUNCTION WITH FORMULAS FOR THRESHOLD VISION AND LIGHT AND DARK ADAPTATION AT THE FOVEA[1]

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I. In a recently published paper (1) the writers have proposed a theory of the functions of the retina which was based upon a large series of tests of threshold vision at the fovea.

The test objects used for the investigations consisted of white squares on a black background, and of white squares with inner black squares as test objects with internal contrast. The total area of each square varied between 81 and 1600 sq. mm. (9 mm. to 40 mm. objects). At the distance from which the test objects were viewed, they subtended visual angles of from 8′ to 36′, and were therefore well within foveal vision. The test objects were illuminated by red light, and were viewed from a distance of 3.75 meters through a red filter (Wratten F 29).

For the white squares (9 to 40 mm.) used as test objects, the product of the cube root of the area multiplied by the light intensity required for vision at the threshold, was found to be a constant,—i.e. $\sqrt[3]{WA} \times I = C$.[2] For square test objects whose total area was 1600 sq. mm., which consisted of an outer white and an inner black area, the product of the square root of the white area and the intensity of the threshold light—$\sqrt{WA} \times I$—was found to be a constant. For square test objects of 9 mm. with internal contrast (total area 81 sq. mm.), the product of the white area and the light intensity—$A \times I$—was a constant.

For all of the test objects at the threshold, both white squares and squares with internal contrast, we found that $\sqrt[3]{WA} \times I - \left(I \times \frac{\sqrt[3]{BA}}{\sqrt[3]{WA}}\right) = C$. In this equation, $\sqrt[3]{WA} \times I$ expresses the amount of light energy which is effective at the ganglion cells of the retina from a

[1] Aided by a grant for brain tumor research from the John and Mary R. Markle Foundation.

[2] The following symbols are used: $T.A.$ = total area; $W.A.$ = white area; $B.A.$ = black area; I = light intensity (in foot candles); T = time in seconds; $T.D.A.$ = time required for dark adaptation (in seconds); C = constant.

white square test object, and $\left(I \times \frac{\sqrt[3]{BA}}{\sqrt[3]{WA}}\right)$ the reciprocal inhibitory effects of the white on the black and the black on the white area of a test object with internal contrast. The formula $\sqrt[3]{WA} \times I - \left(I \times \frac{\sqrt[3]{BA}}{\sqrt[3]{WA}}\right)$ yielded a constant figure for each individual for all of the test objects that were used,—both white squares and white squares with inner black squares as test objects with internal contrast.[3]

The formula was, therefore, a mathematical expression of the functional relationships of the cones, bipolar cells and ganglion cells of the retina. It could be used to express the amount of effective light energy that was transmitted to the brain and that was necessary for the recognition of an object illuminated by the light intensity necessary for vision at the threshold.

II. *The effective light energy which is transmitted to the brain by the light adapted eye.* For the experiments described in this section, the test objects consisted of 1, a white square of 40 mm., and 2, a square with internal contrast, whose total size was 80 mm. The total area of the latter was 6400 sq. mm., of the outer white area 1600 sq. mm. and of the inner black area 4800 sq. mm.

For each series of tests, the object and intensity of illumination were the same, but the intensity of the bright light and the duration of the exposure to it were varied. All of the tests were made with the two eyes. After the exposure of the eyes to the bright light, the time which elapsed before the test object could again be seen, was considered to be the time required for dark adaptation. Therefore the length of the refractory period was used as a measure of the effect of the bright light.

For these studies of dark adaptation we made use of a visual box, by means of which one or both eyes could be exposed to a bright light of any intensity for any desired time. The apparatus and procedure have been fully described in an already published paper (2). The steps of the procedure for the study of dark adaptation consisted of:

1. The establishment of the light required for vision of the test object.
2. The exposure of the eyes to bright light of a known intensity for a measured period of time.
3. The establishment of the time which elapsed until the test object could again be recognized.

Therefore we studied threshold vision at only a small part of the dark adaptation curve.

We were able to discover a simple equation for light adaptation, as an expression of the energy transmitted to the visual centers by the exposure of the eyes to a bright light.

According to the Bunsen-Roscoe law, within certain limits, the photochemical effect produced by light is equal to the product of the intensity

[3] The conditions under which this statement is correct are described in detail and the test objects are illustrated in papers published in the Bulletin of the Neurological Institute of New York, 1937, vi, no. 2.

of the light and the time during which it acts. In other words, a given photochemical effect is produced by a given number of vibrations independent of the time required to receive them. According to this law,

TABLE 1

The relation between $I \times T$ and the time required for dark adaptation

		I	T	$I \times T$	TIME REQUIRED FOR DARK ADAPTATION	
					C. A. E.	H. S.
		foot candles	*seconds*		*seconds*	*seconds*
A	$I \times T = 50$	1.25	40	50	7.3	12.1
		1.60	30	50	4.6	8.7
		2.50	20	50	3.2	6.7
		5.00	10	50	2.5	4.0
						J. S.
B	$I \times T = 200$	3.3	60	200	11.4	10.9
		6.6	30	200	9.5	7.4
		10.0	20	200	6.9	7.2
		20.0	10	200	4.9	5.0
						H. S.
C	$I \times T = 400$	6.6	60	400	23.5	28.6
		13.2	30	400	14.1	15.4
		20.4	20	400	11.1	12.6
		40.0	10	400	6.7	8.9
D	$I \times T = 600$	10	60	600	25.3	29.7
		20	30	600	15.8	23.4
		15	40	600	13.9	17.8
		60	10	600	10.5	14.3
						J. S.
E	$I \times T = 1{,}200$	20	60	1,200	31.5	24.2
		40	30	1,200	23.3	17.5
		60	20	1,200	16.6	12.9
		80	15	1,200	15.6	10.4
						H. S.
F	$I \times T = 1{,}600$	20	80	1,600	33.4	34.4
		40	40	1,600	23.3	29.4
		80	20	1,600	19.0	21.2
		260	6.2	1,600	7.3	12.0

if $I \times T = C$, the *amount* of light to which the eyes are exposed remains the same.

The formula $I \times T = C$ indicates the amount of light acting on the

retina, but it does not express the resultant energy liberated or the number of impulses sent to the brain. This is shown by the results of the tests in table 1.

These figures show, for any given value of $I \times T$, that if the intensity of light was low but the time of exposure of the eyes to the light was pro-

TABLE 2

The relation between $\sqrt[3]{I} \times T$ and the time required for dark adaptation

		I	$\sqrt[3]{I}$	T	$\sqrt[3]{I} \times T$	TIME REQUIRED FOR DARK ADAPTATION	
						C. A. E.	J. S.
A	$\sqrt[3]{I} \times T = 10$	8	2	5.0	10	4.7	5.7
		27	3	3.3	10	4.5	5.8
		64	4	2.5	10	4.6	5.4
B	$\sqrt[3]{I} \times T = 36$	8	2	18	36	7.3	7.1
		27	3	12	36	7.3	7.5
		64	4	9	36	7.8	7.3
							H. S.
C	$\sqrt[3]{I} \times T = 72$	8	2	36	72	22.1	18.8
		27	3	24	72	22.6	18.8
		64	4	18	72	23.6	19.1
D	$\sqrt[3]{I} \times T = 108$	8	2	54	108	31.4	26.6
		27	3	36	108	30.5	26.2
		64	4	27	108	28.5	23.6
							J. S.
E	$\sqrt[3]{I} \times T = 144$	27	3	48	144	24.3	25.0
		64	4	36	144	24.0	26.3
		80	4 32	33.3	144	24.5	25.1
F	$\sqrt[3]{I} \times T = 180$	27	3	60	180	34.2	35.9
		64	4	45	180	35.9	38.9
		80	4.32	41.6	180	34.6	33.9
G	$\sqrt[3]{I} \times T = 216$	64	4	54	216	34.7	41.0
		80	4.32	50	216	33.3	39.8
		286	6.6	32.7	216	75.8 (a)	47.3 (a)

longed, the duration of the refractory period was longer than when the relation between time and intensity was reversed. The important result of these tests was the demonstration that the Bunsen-Roscoe law $I \times T$ expresses the amount of light that is effective at the retina but not the energy that is transmitted to the brain for vision.

A series of investigations, a few of which are given in table 2, showed that (within the limits of experimental error) when the product of the *cube root* of the intensity of the bright light and the time of exposure to it ($\sqrt[3]{I} \times T$) was a constant, the time required for dark adaptation of any individual was also constant.

These tests showed that when $\sqrt[3]{I} \times T = C$ the time required for dark adaptation was constant, excepting in one instance (G (a)) in which the light was of great intensity *and* the time of exposure to it was long. In this one instance some other factor must enter.

III. *A formula for dark adaptation at the fovea.* It is well known that the time required for dark adaptation is dependent upon at least four factors: 1, the size of the test object; 2, the intensity of its illumination; 3, the intensity of the bright light to which the eyes were previously exposed, and 4, the *duration* of the exposure to the bright light. The other factors were constant in the tests.

In the first section of this paper we have indicated that the effective light energy which is transmitted to the brain by the retinal ganglion cells may be expressed by the formula $\sqrt[3]{WA} \times I - \left(I \times \frac{\sqrt[3]{BA}}{\sqrt[3]{WA}}\right) = C$ and this constant may be called *C1*. In the second section it was shown that the energy from a bright light, effective at the retinal ganglion cells and by them transmitted to the visual centers may be expressed by the formula $\sqrt[3]{I} \times T = C$, a constant which we shall call *C2*.

The more intense the bright light or the longer the eyes are exposed to it (the other factors being constant), the larger will be the value of *C2*, and the *longer* will be the time required for dark adaptation. On the other hand, the larger the size or the more intense the illumination of the test object (i.e., the larger *C1*) (the other factors being constant), the *shorter* will be the time required for dark adaptation. Therefore there is an inverse relationship between *C1* and *C2* and it was found that this relationship was expressed by the fraction $\frac{C1}{\sqrt[3]{C2}}$. A large series of tests demonstrated that $\frac{C1}{\sqrt[3]{C2}} \times \sqrt{TDA} = C$. The complete formula for dark adaptation is therefore:

$$\frac{\sqrt[3]{WA} \times I' - \left(I' \times \frac{\sqrt[3]{BA}}{\sqrt[3]{WA}}\right)}{\sqrt[3]{\sqrt[3]{I''} \times T}} \times \sqrt{TDA} = C$$

in which I' is the intensity of illumination of the test object, and I'' the intensity of the bright light.

This constant was called *C5* and it was derived from the following calculations:

1. A large series of tests showed us that $C1 \times \sqrt{TDA}$ gave a constant which we called $C3$, as given in table 3.

2. Furthermore, we found that $\frac{\sqrt{TDA}}{\sqrt[3]{C2}}$ gave a constant (which was called $C4$) as given in table 4.

The formulas for $C3$ and $C4$ show that $C1$ is inversely related to $\sqrt{TDA}$ and that $\sqrt[3]{C2}$ is directly related to $\sqrt{TDA}$. Both of these relationships give constants. Therefore the formula $\frac{C1}{\sqrt[3]{C2}} \times \sqrt{TDA}$ must also give a constant,—which is the constant we have called $C5$.

TABLE 3

Two eyes 40 mm. white square. Duration of stimulation by bright light—5 seconds. In test 1, the test object was illuminated by the light intensity necessary for threshold vision; in each succeeding test, the illumination of the test object was increased by 10 per cent.

	$C1$	TDA	$\sqrt{TDA}$	$C1 \times \sqrt{TDA}$
1	50.90	13.5	3.57	181.71
2	55.98	10.5	3.25	181.95
3	61.07	7.4	2.73	166.74
4	66.16	5.8	2.4	158.80
5	71.25	5.1	2.25	160.32
6	76.34	4.8	2.2	167.96
7	101.79	3.4	1.87	172.54

TABLE 4

Two eyes, 40 mm. test object; intensity of illumination of test object, threshold plus 20 per cent; duration of exposure to bright light of 80 foot candles, 5, 10, 15 and 30 seconds.

	$\sqrt[3]{C2}$	TDA	$\sqrt{TDA}$	$\frac{\sqrt{TDA}}{\sqrt[3]{C2}}$
1	2.78	12.8	3.6	1.29
2	3.51	20.3	4.5	1.28
3	4.0	29.3	5.4	1.35
4	5.05	37.2	6.1	1.21

This constant (and the formulas from which it is derived) is a mathematical expression of the energy transmitted to the visual centers in the brain and of the phenomenon of dark adaptation.

The equation was applied to series of visual tests of dark adaptation (TDA) in which 1, the size of the test object; 2, the illumination of the test object; 3, the intensity of the bright light, or 4, the duration of exposure to the bright light (light adaptation) was varied. Examples are given in tables 5 to 8.

TABLE 5

Four test objects. Illumination of T.O. 7.6 foot candles. Intensity of bright light 80 foot candles. Duration of each exposure to bright light, 10 seconds.

	TOTAL AREA	WA	BA	ILLUMINATION	$\sqrt[3]{WA}$	$\sqrt[3]{BA}$	$C1$	$C2$	$\sqrt[3]{C2}$
				foot candles					
1	1,600	400	1,200	7.6	7.37	10.6	45.14	43.20	3.51
2	1,600	800	800	7.6	9.3	9.3	63.08	43.20	3.51
3	1,600	1,200	400	7.6	10.6	7.37	75.24	43.20	3.51
4	1,600	1,600		7.6	11.7		88.92	43.20	3.51

	$\frac{C1}{\sqrt[3]{C2}} \times \sqrt{TDA}$	TIME REQUIRED FOR DARK ADAPTATION	
		Found by tests	Calculated by formula
		seconds	*seconds*
1	53.76	17.6	16.81
2	50.12	7.6	8.4
3	54.35	6.4	5.9
4	50.35	3.9	4.2
Average.....	52.14		

TABLE 6

Forty millimeter white square as test object. Tests begun with threshold light, and in each succeeding test the illumination of the object was increased by 10 per cent. Intensity of bright light 80 foot candles. Duration of exposure to bright light 5 seconds.

	$C1$	$\sqrt[3]{C2}$	TDA	$\sqrt{TDA}$	$\frac{C1}{\sqrt[3]{C2}} \times \sqrt{TDA}$
1	50.89	2.78	13.5	3.68	67.34
2	55.98	2.78	10.5	3.25	65.33
3	61.07	2.78	7.4	2.73	59.79
4	66.16	2.78	5.8	2.4	57.10
5	71.25	2.78	5.1	2.25	57.60
6	76.34	2.78	4.8	2.2	60.28
7	101.79	2.78	3.4	1.87	68.44
Average.....					62.27

	TIME REQUIRED FOR DARK ADAPTATION	
	Found by tests	Calculated by formula
	seconds	*seconds*
1	13.5	11.56
2	10.5	9.55
3	7.4	8.06
4	5.8	6.76
5	5.1	5.9
6	4.8	4.84
7	3.1	2.89

TABLE 7

Test object with contrast. *TA* 6400 sq. mm. *WA* 1600. *BA* 4800. Intensity of illumination 6.21 foot candles. Intensity of *and* duration of exposure to bright light varied in each test.

	TDA	*T*	$\sqrt[3]{I}$	$\frac{\sqrt[3]{I} \times T}{(C_2)}$	$\sqrt[3]{C_2}$	*C1*	$\frac{C1}{\sqrt[3]{C_2}}$
		seconds					
1	3.3	60	1.49	93.40	4.48	62.59	14.0
2	6.6	30	1.88	56.40	3.76	62.59	16.6
3	10.0	20	2.16	43.20	3.46	62.59	18.1
4	20.0	10	2.72	27.20	3.00	62.59	20.8

	I	$\sqrt{TDA}$	$\frac{C1}{\sqrt[3]{C_2}} \times \sqrt{TDA}$	TIME REQUIRED FOR DARK ADAPTATION	
				Found by tests	Calculated by formula
1	10.9	3.3	46.20	10.9	10.90
2	7.4	2.72	45.15	7.4	7.84
3	7.2	2.66	48.14	7.2	7.60
4	4.99	2.24	46.59	4.99	4.97
Average			46.52		

TABLE 8

Test object with contrast. *TA* 6400 sq. mm. *WA* 1600. *BA* 4800. Intensity of illumination of test object 5.4 foot candles. Intensity of bright light 40 foot candles. Duration of exposure to bright light varied: (1) 5 seconds (2) 10 seconds (3) 15 seconds (4) 20 seconds (5) 25 seconds (6) 30 seconds.

	C1	C_2 $(\sqrt[3]{I} \times T)$	$\sqrt[3]{C_2}$	$\frac{C1}{\sqrt[3]{C_2}}$	*TDA*	$\sqrt{TDA}$
1	55.40	17.10	2.57	21.5	7.0	2.64
2	55.40	34.20	3.25	17.0	9.9	3.15
3	55.40	51.30	3.72	15.0	12.9	3.59
4	55.40	68.40	4.1	13.5	15.3	3.91
5	55.40	85.50	4.4	12.6	19.9	4.47
6	55.40	102.60	4.68	11.8	23.0	4.8

	$\frac{C1}{\sqrt[3]{C_2}} \times \sqrt{TDA}$	TIME REQUIRED FOR DARK ADAPTATION	
		Found by tests	Calculated by formula
1	56.76	7.0	6.55
2	53.55	9.9	10.43
3	53.85	12.9	13.47
4	52.79	15.3	16.0
5	56.32	19.9	19.1
6	56.64	23.0	21.7
Average	54.98		

COMMENT. 1. It must be remembered that the tests upon which the equations are based were made with red light, and therefore that the conclusions are applicable only to red light. It is probable, however, that tests with other monochromatic or with white light will give similar if not identical results. A small number of tests with white light, which we have made, point in that direction.

2. Our studies deal entirely with foveal vision, i.e., the rod-free part of the retina. It is probable, however, that similar tests made of vision of the entire retina or of the periphery, will give similar results. Under the latter circumstances, on account of the presence of rods in addition to cones, and of the different effects of light upon these two sets of receptors, the equations might have to be modified for peripheral vision. On the other hand, it may well be that the equations will hold equally well for all parts of the retina.

3. There are some limitations to the formula for dark adaptation to which attention must be called. The formula does not hold for very bright light (above 286 foot candles) if the duration of exposure is long. Under these conditions some other factor is added. Furthermore, the lowest limit at which the formula holds is uncertain because with our simple apparatus it was not possible to measure very slight differences of low intensities of light.

4. The constants obtained in different series of tests on the same individual on different days, will often vary. This is in accordance with the well known fact that the reactions of the nervous system of an individual vary diurnally to some extent,—no matter what the character of tests that are made. Thus if quantitative tests of acuity of sensation are made on different days, there will be some variations in the results. This fact is well known, and in our tests of the special senses,—smell, hearing and vision—we have found this to be the case. But if the calculations by the equations are based upon the reactions of the individual on any one day, all of the constants will be found to be the same for the same individual.

DISCUSSION. The results described in this report appear to indicate that it is possible to express in mathematical terms the effects of light upon the eye and the brain which produce conscious vision. The formulas are mathematical expressions of the energy produced by light, and of the relative value for conscious vision, of different degrees of light stimulation. In effect, therefore, the result is a physical expression of cerebral activity.

In his classic paper "Zur Theorie des Elektrischen Reizes," Nernst (3) foreshadowed these results. He stated that in organic tissues electrical threshold stimuli could cause only changes in the ionic concentrations of

substances. It was possible to express such changes causing sensation in a mathematical physical formulation. The experimental data covered periods of time of less than one second. He anticipated that further experiments involving longer periods of time might some day give similar results.

According to Nernst's theory, the threshold effect upon the nerve cell is based upon a simple square root relation, i.e., $I \times \sqrt{T} = C$. The results of the studies of dark adaptation described in this paper have led to a similar conclusion: the effect upon the brain, as indicated by the duration of the time required for dark adaptation, has a square root relation to the energy which reaches the brain from the ganglion cells of the retina. This is indicated by the formula $\frac{C\,1}{\sqrt[3]{C\,2}} \times \sqrt{TDA} = C$. From experiments that will be described in another paper, it appears probable that there is a similar square root relation between other impulses that reach the cells of the brain and the effects there produced.

The cube root relations, evidenced by the formula for *C 1* and the formula for *C 2*, appear to have a relation to the functions of the retinal ganglion cells and are in accord with the theory of the functions of the retina described in a paper already published (1).

The important fact that in the light-adapted eye, the *effect* of bright light does not obey the Bunsen-Roscoe law, needs some consideration. The fact that the effect of light is expressed by the equation $\sqrt[3]{I} \times T = C$ instead of $I \times T = C$ is not a contradiction to the law, but a demonstration that the nerve energy produced by an amount of light is not directly proportional to the quantity of light or the photochemical effect. The statement of Hecht (4) that the effect of light upon visual purple and the change from visual purple to visual yellow obeys the Bunsen-Roscoe law, may be entirely correct. From this one cannot draw the conclusion that the resultant nervous energy that is released to the brain is governed by the same law.

SUMMARY

1. Formulas are given for threshold vision and for light and dark adaptation at the fovea.

2. The formula which gives the constant we have called *C 1* is a mathematical expression of retino-cerebral functions associated with vision.

3. The formula which gives the constant we have called *C 2* is a mathematical expression of the energy produced during light adaptation and a measure of the influence of bright light upon the duration of the refractory period (dark adaptation).

4. The formula for dark adaptation is a mathematical expression of cerebral activity for conscious threshold vision as measured by the duration of the refractory period (dark adaptation).[4]

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[4] It is hardly necessary to mention that the so-called constants are only constant under certain experimental conditions. For example, C 1 is a constant expressing the relationship of area and intensity for vision at the threshold in the dark adapted eye. In the formula for dark adaptation, it may be a variable dependent upon the factors which influence dark adaptation. In the formula for dark adaptation, C 2 is a constant which expresses the relationship of time and intensity during light adaptation, only when the other factors which influence dark adaptation and the time required for dark adaptation are constant. Otherwise C 2 is a variable.

THE LOCATION OF THE CARDINAL ANATOMICAL ORIENTATION PLANES PASSING THROUGH THE CENTER OF WEIGHT IN YOUNG ADULT WOMEN[1]

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The early theoretical and experimental contributions to the study of the center of gravity in man have been reviewed by Haycraft (Schäfer, 1900), Luciani (1915) and Steindler (1935). Although the diagnostic value of the gravity line in the physical examination of posture has been universally recognized, its experimental determination is neglected in the majority of the many tests devised for the objective evaluation of stance. Few data are available, particularly on the female, whose bodily configuration differs from that of the male in ways which influence the location of the center of weight. The first extensive observations on young adult women were concerned with the height of the center of gravity and were made in this laboratory by Dawson and his co-workers (Croskey et al., 1921). They used a modification of the simple method originally described by Borelli (Schäfer, 1900). In 1931 Cotton published his more accurate Differential Method and extended the observations of Dawson. Our object has been to locate the three cardinal orientation planes passing through the center of weight and to establish their range of variability in this sex and age group.

METHODS. *1. The location of the horizontal plane passing through the center of gravity.* The apparatus illustrated in figure 1 permits direct measurement of the height of the center of gravity in a single simple manipulation. A heavy plank balance board was suspended by a metal yoke between two supporting knife edges. Rubber tired casters were mounted near its extremities. Upon these rested an unyielding wooden platform moved by a crank shaft attached to a differential gear which simultaneously actuated a counter-weight running on ball bearing wheels over a steel track. Any movement of the platform, displacing the center of gravity and thus affecting its importance in producing rotation about the supporting knife edges is exactly balanced by a movement of the counter-weight in the opposite direction. The subject assumes a standardized position upon the adjustable platform. When balanced, the center of

[1] Supported in part by a grant from the Wisconsin Alumni Research Foundation.

gravity of the recumbent body lies in a vertical plane passing through the supporting knife edges.

2. The location of the cardinal vertical orientation planes passing through the center of gravity. The approach was that of Reynolds and Lovett (1909). The subject stands upon a horizontal beam supported at each end on steel knife edges, one of which rests upon a platform scale equipped with a distant reading dial. Since the sum of the counter forces of gravity is equal to the total weight, and since the center of weight divides the support into parts of a length in inverse proportion to the intensity of the reactions at the knife edges, the distance of the concentrated load from the posterior support may be determined by equating moments. To locate the coronal and sagittal planes passing through the center of weight, photographs of the subjects were taken on photostat paper in profile and

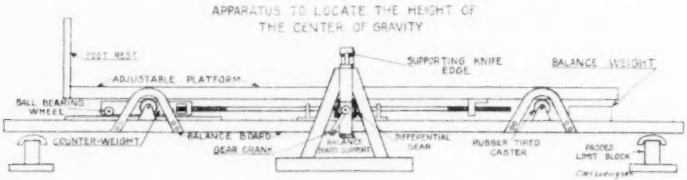

Fig. 1. The entire system, swinging freely from the supporting knife edges, is first adjusted by moving the balance weight until the moments on either side of the axis of rotation are equalized. The balance board then remains in equilibrium irrespective of the position of the adjustable platform. The center of gravity of the apparatus when sustaining a recumbent subject lies below and close to the knife edges which bear the weight of the aggregate. Tipping due to unequal loading raises the center of gravity. The balance board tends always to seek the horizontal, enhancing the ease of equilibration. The influence of a ten gram weight at the end of the balance board may be detected with a spirit level.

back view respectively. A signal lamp in circuit with the camera shutter indicated the instant of photographic exposure. The mean position of the scale indicator was then read. A vertical line passing through the center of weight was introduced into the photographs after correcting its calculated distance from the posterior knife edge for variable degrees of paper shrinkage in drying.

The subjects of the investigation were healthy young adult women, 357 college freshmen and 88 graduate and undergraduate professional students in physical education. The subjects were either nude or clothed in a dry tank suit. Center of gravity determinations were made without regard to meals or time of day since the work of Dawson and Cotton had demonstrated these to be negligible sources of error. Distortions attributable to exercise circulatory readjustments did not occur. For the location of the vertical orientation planes two attitudes were studied.

The college freshmen took their best standing postures. The professional students assumed a confortable natural stance.

RESULTS. Table 1 contains the essential data concerned with the location of the height of the center of gravity. The findings are in close agreement with those of Dawson and Cotton. In spite of marked variance in tallness and weight, the percentage height of the center of gravity departs from the mean over a remarkably small range, the scatter amounting to less than 1 cm. The average weight of the 88 professional students was 58.370 ±0.493 kgm. with a standard deviation of 6.851 ±0.348. It ranged from 45.645 to 78.761 kgm., but there was no statistically significant relation of the per cent height of the center of gravity to this variable indicative of concentration of weight about the hips and thighs in the heaviest subjects. The average height was 1.644 ±0.004 meter with a

TABLE 1

Per cent of total height below the center of gravity

	COLLEGE WOMEN	PROFESSIONAL STUDENTS	COTTON'S DATA	DAWSON'S DATA
Number of subjects	357	88	38	50
Mean height of center of gravity	55.174	55.195	56.07	55.44
P.E. mean	0.030	0.056	0.07	0.096
Minimum	53.001	53.295	54.70	53.5
Maximum	59.005	57.788	57.30	58.5
Standard deviation	0.835	0.775	0.67	1.003
P.E. of standard deviation	0.021	0.040	0.05	0.058
Median	55.137	55.136	56.17	55.39
Lower quartile	54.609	54.714	55.56	54.69
Upper quartile	55.704	55.699	56.63	56.12
Semi-quartile range	0.548	0.492	0.53	0.72
Coefficient of variability	1.51	1.41	1.69	1.80

standard deviation of 0.058 ±0.003. Tallness was not associated with a disproportionately high center of weight. These subjects were of good physique, the dispersion about the mean percentage height of the center of gravity being roughly equivalent at all statures. The average age was 20.2673 ±0.1299 years with a root-mean-square deviation of 1.776 ±0.0918. Since it ranged from 17 to 26 years, the per cent height of the center of gravity was also plotted against this variable. It demonstrated no trend toward the development of a bodily contour significantly altering the distribution of mass with increasing maturity. That this constancy in the percentage height of the center of gravity is significant and not due to an unusual homogeneity in type of build among professional physical education students is suggested by the close agreement between the experimental findings on this and the general college group.

Steindler (1935) places the line of gravity approximately 4 cm. in front of the ankle joint. Although our average findings approach this estimate, the data presented in table 2 indicate that the weight line may be located over an exceedingly wide range along the antero-posterior foot support, varying from 0.388 to 13.536 cm. in front of the ankle joint. The average location of the center of gravity as projected into the base of support of women standing in their best posture was 4.928 $\pm$0.073 cm. anterior to the external malleolus with a standard deviation of 1.95 $\pm$0.0519. The dispersion about the mean is great, the location of the center of gravity in this plane being 26 times more variable than its percentage height. The mean distance of the weight line from the malleolus was 14 per cent greater when a comfortable natural stance was assumed. However, consideration of the relation of the gravity line to the malleolus without regard to foot length, as is customary, leads to errors in interpretation. The mean foot length of the average professional physical education

TABLE 2

Distance of the weight line from the external malleolus

	COLLEGE WOMEN	PROFESSIONAL STUDENTS
Number of subjects	327	87
Mean distance from malleolus (cm.)	4.928	5.650
P.E. mean	0.073	0.130
Minimum	0.388	1.272
Maximum	13.536	9.388
Standard deviation	1.95	1.80
P.E. of standard deviation	0.0519	0.09
Coefficient of variability	39.5	31.8

student was greater than that of the college freshman so that the center of gravity occupied almost identical positions when projected into the base of support, 40.04 and 40.09 per cent of the antero-posterior underprop diameter being dorsal to the gravity line in the natural and best stance respectively. Disregarding the toes, which usually bear no weight in normal stance, places the mean center of gravity in a pivotal position with roughly equivalent diameters of structural support fore and aft.

The weight line was equidistant between the heels in only 10.98 per cent of the college girls standing in their best posture and in 13.25 per cent of the physical education students assuming a comfortable stance. In the majority the weight was unequally distributed between the feet, falling preponderantly to the left in the natural stance. This may be an expression of handedness, stance being a position of readiness for movement in which the right side tends to swing forward first, or a posture of preparedness, the left foot bracing to meet dominantly right sided activity of the

non-weight-bearing limbs. When a conscious effort was made to stand in a good posture, the position assumed by the college freshmen, the weight line fell preponderantly to the right. This may be the unconscious overcorrection of a natural tendency to attain balance by holding the weight on the less developed side.

Steindler (1935) believes that in the majority of human subjects "there is a time in life when apparently complete and stable symmetry of the body exists," curvatures of the spine only following upon the habitual assumption of asymmetrical bodily attitudes. In his definition of "normal posture" the gravity line intersects the head in the mid-occipital region, coincides with the vertebral column, passes through the gluteal fold and is equidistant from the heels. The existence of such a posture even in young adults must be a theoretical abstraction. The one-sided preponderance of habitual movement patterns and occupational postures suggests on *a priori* grounds that their equilibratory compensation will automatically induce attitudinal anomalies giving rise to a physiologically normal posture characterized by a frank asymmetry in configuration. Our experimental findings bear this out.

SUMMARY AND CONCLUSIONS

The three anatomical orientation planes passing through the center of weight were located in 445 young adult women. The height of the horizontal plane above the base of support was measured in recumbency. The vertical orientation planes were determined in the upright stance when a natural, comfortable posture was assumed or when the subject stood in her so-called "best posture." The evidence justifies the following conclusions:

1. The mean percentage height below horizontal plane passing through the center of gravity is 55.

2. Departures from the mean percentage height of the center of gravity vary over a singularly small range in spite of wide disparity in tallness and weight.

3. The average location of the line of gravity is 5.08 cm. in front of the ankle joint.

4. It varies widely from a position nearly coincident with the axis of rotation of the ankle joint to one at the extreme limits of the anteroposterior foot support.

5. The upright stance is asymmetrical in the majority of young adult women, the weight falling preponderantly to the left in a comfortable stance and to the right when the "best posture" is assumed.

6. It is suggested that the transverse shift in the center of weight associated with voluntary correction is an overcompensation for a natural

tendency to balance unequal development incidental to handedness, by standing on the weight bearing limb of the weaker side.

ACKNOWLEDGMENTS. Our thanks are due Mr. James Hipple, mechanician of the State of Wisconsin General Hospital, for his contribution to the development of the tilting board used for the determination of the height of the center of gravity; to Professors Trilling and Bassett for placing the posture study photographic facilities of the Department of Physical Education at our disposal; to Professor Ingraham and the Computing Service of the Mathematics Department for the statistical handling of the data.

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STANDING AS A GEOTROPIC REFLEX

The Mechanism of the Asynchronous Rotation of Motor Units[1]

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It has been said that man is a made-over animal (Hooton, 1936). The greatest modifying force to which he has been subjected in his evolution to bipedism is gravity. Posture is an automatism controlled by geotropic reflexes which Fulton and Sherrington (1930) date as pre-human in antiquity. The receptors in this neuron circuit first described by Liddell and Sherrington (1924) must be stimulated by every shift in the center of gravity. Our observation of the extreme variability in the location of the center of weight in the vertical orientation planes of a homogeneous group of young adults studied by a method involving instantaneous photography, suggests that the upright stance may be associated with degrees of postural sway which markedly alter the tone demanded of the antigravity muscles for the equilibration of rotatory stresses effective at the joints of the vertebral column and the weight bearing limbs. The efforts to explain the peripheral mechanism of tonus have been largely hypothetical (Barbour and Stiles, 1912; Forbes, 1922; Fulton and Liddell, 1925). The object of this study was to determine the magnitude of involuntary center of gravity shifts during the assumption of a natural vertical stance, and to evaluate their importance as effective stimuli for the myotatic reflex.

Methods. The method, based on Newton's Third Law of Motion, was the same in fundamental detail as that already described for the determination of the vertical orientation planes passing through the center of gravity (Hellebrandt et al., 1938). A series of platforms was constructed at right angles to each other to permit concurrent registration of the center of gravity in the antero-posterior and transverse planes, and changes in partial weight were graphically recorded as a constant moving kymogram. To project the center of gravity into the base of support it was only necessary to fix the position of the feet in respect to the knife edges of the two platforms and establish a kymographic base line. After calibration of the instrument, the latter permits the conversion of every oscillation of the center of gravity into a change in partial weight, and by equating moments,

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the center of gravity may be simultaneously established in the two vertical orientation planes. A removable foot guide was placed upon the uppermost platform for the standardization of the foot posture in Morton's (1935) position of greatest stability in stance. In this footing the lateral diameter of structural support approximates the antero-posterior, the toes are turned out and the feet form an angle of 30 to 40 degrees. A piece of absorbent paper was also placed upon the platform. The subject stood in a concentrated solution of potassium permanganate and then carefully placed the moist feet in position with the heels touching the posterior ledge and the separating block of the foot guide, the fifth metatarso-phalangeal joints abutting against the lateral restraints. This provided a durable

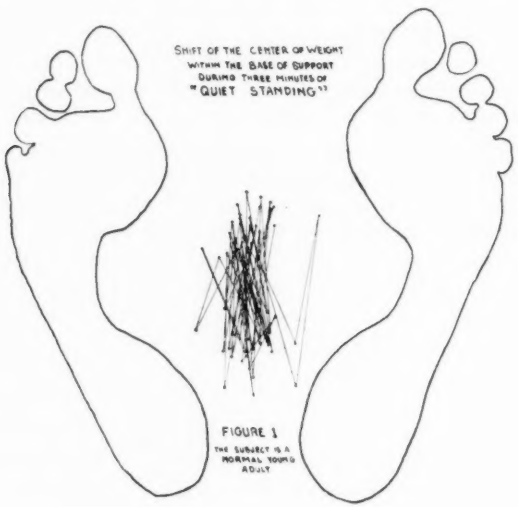

FIGURE 1
THE SUBJECT IS A NORMAL YOUNG ADULT

imprint of the area of under-propping and established lines of reference a known distance from the points of support in terms of which the center of gravity was mathematically located. All observations were made with the subject standing in a natural comfortable stance for three minutes during which the gaze was fixed upon a designated point, giving always the same visual stimulation.

RESULTS AND THEIR DISCUSSION. Repeated observations were made on a group of ten healthy vigorous young adult women. Many experiments have been performed under normal conditions and in association with various experimentally produced postural and equilibratory disturbances. It has long been known that sway is inseparable from the upright stance,

but its magnitude in terms of shifts in the center of weight has neither been experimentally determined nor related to the margins of static security furnished by the base of support. *The center of weight shifts incessantly.* In a very steady subject its rhythmical oscillations do not exceed one centimeter. They may, however, be strikingly large in a vigorous subject with good kinesthesia and equilibratory sense. Figure 1 is a reproduction of the typical response of such a subject showing a high degree of involuntary reciprocating motion during the maintenance of a vertical stance. Interference, by voluntary effort, with the natural tendency to sway reduces the magnitude of the shift in the center of weight, but it cannot be entirely controlled. The subject trained to avoid interference by cerebral activity, shows a very characteristic and consistent oscillatory pattern upon repeated observation when he stands "quietly," undisturbed by extraneous sensory stimuli. Standing must be looked upon as a dynamic phenomenon. It is in reality movement upon a stationary base.

Postural sway is always greater in the antero-posterior than in the transverse vertical plane. A plumb dropped from the center of gravity of a standing subject falls, on an average, 5 cm. in front of the ankle joint (Hellebrandt et al., 1938). Hence, gravity is a force which constantly places a stretch upon the extensors of that joint. Its concurrent effect upon the quadriceps and the gluteus maximus depends upon the relation of the gravity line to the axes of rotation of the knee and hip joints. According to the teachings of Sherrington, stretch brings about a reflex contraction confined to the proportion of fibers influenced by the stimulus. By thus equilibrating the gravitational stress, the stretch is relieved. As soon as it ceases, contraction also gives way. The multijointed biped then totters because of the mechanical instability of the orthograde stance with its very high center of gravity and relatively small base of support. In consequence, new sets of stretch afferents are constantly stimulated by the incessant shifting of the center of weight. We may thus account for the asynchronous rotation of motor units which gives to postural contraction its unique indefatigability. It seems highly logical that the automatic control of postural contraction should find its explanation in postural sway.

CONCLUSIONS

1. Standing is in reality movement upon a stationary base, sway being inseparable from the upright stance.

2. The incessant shifting of the center of weight during stance constantly stimulates new stretch afferents, and hence may account for the asynchronous rotation of motor units which characterizes postural contraction.

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THE INFLUENCE OF CALCIUM AND POTASSIUM UPON INTESTINAL ABSORPTION

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Using isolated segments of small intestine from the rabbit, Magee and Sen (1931) studied the absorption of glucose and xylose when the segment was immersed in sugar-free Tyrode solution and when calcium was withheld from the Tyrode solution. In the presence of Ca, glucose passed into the bath more rapidly than xylose, but this result was reversed when the bath contained no Ca. This indicated that selective absorption is dependent upon Ca-ions in the bath. Later Magee and Sen (1932) reported that parathyroidectomized rats with blood Ca below normal absorbed less glucose than normal rats. When Ca was added to the glucose these rats absorbed at a normal rate. When $CaCl_2$ (0.32 per cent) was added to the glucose given to normal rats absorption was depressed but a lower $CaCl_2$ addition (0.02 per cent) stimulated absorption. The absorption of glucose by rabbits with low blood Ca was no different from that of normal rabbits. Gellhorn and Skupa (1933) perfused frog's intestine with 3.15 per cent glucose solution and the intestinal vessels with Ringer solution. They found that small amounts of KCl markedly increased while $CaCl_2$ decreased permeability of the gut to glucose. McDougall (1935) has shown that rats reared on a diet that caused a low serum Ca absorbed isotonic solutions of glucose, xylose and sorbose at the same rate as normal rats. The sugar solutions were injected directly into the small intestine in urethanized animals.

The above observations show so much disagreement that it seemed desirable to study further the effects of K and Ca on the absorption of glucose. The work here reported was done on dogs with chronic closed loops of small intestine, made essentially as described by Burget (1934). These animals were in good condition and kept on a constant diet. As a supplement to the work on dogs a series of experiments was carried out on normal rats.

PROCEDURE. The closed loop method has been described in detail by Burget, Moore and Lloyd (1932). The closed loop was always washed clear of debris with warm physiological salt solution before a ten per cent solution of dextrose was injected. After one hour the remaining sugar solution was withdrawn from the loop with a syringe and the loop again

washed with warm saline. The washings were added to the sugar solution, which had been withdrawn, and the whole was diluted to 100 cc. in a volumetric flask. A one to fifty dilution of this was used for sugar determination by the Shaffer-Hartman method. When indicated, the dilution was altered to bring the final reading in cubic centimeters of thiosulphate used for titration within the limits of 12 to 18 cc. since this has been shown to be the amount of sugar most accurately determined by this method.

In using normal rats the procedure described by Cori (1925) was followed. The last food was given forty-eight hours before the experiment, but water was left in the cage throughout. The cages had screen bottoms that allowed feces to drop out of the reach of the animals. The weights of rats varied between 130 and 270 grams. By means of a no. 10 French catheter 2 cc. of a 50 per cent solution of the sugar was introduced into the stomach. At the end of one hour the animal was killed by a blow on the head. The abdomen was immediately opened and ligatures placed around the esophagus just above the cardia and around the ileum near the ileocolic valve. It was found unnecessary to include the lower bowel. After removal, the stomach and small intestine were washed out by forcing 50 cc. of warm water through them. After making fluid contents and washings up to 100 cc. the amount of the sugar was determined. Thus the quantity of sugar absorbed was obtained by subtracting the amount removed from the amount injected. Pfanstiehl dextrose with a specific rotation of $+52°$ was used throughout.

Results. The results of the series of experiments on seven dogs are shown in table 1. It will be seen that the potassium salt produces a definite increase in the amount of glucose absorbed from the intestine in one hour while calcium causes a decrease in the amount absorbed. The increase in absorption produced by the presence of 0.1 per cent potassium chloride may be seen to be approximately equal to the decrease occasioned by a similar concentration of the calcium salt when compared to the rate of absorption of the pure sugar solution in each individual dog. Due to slight anatomical variations in the intestine in all animals it is impossible to make the loops of a constant size. This will account in large degree for the individual variations in the amount of sugar absorbed in one hour when injected in pure solution. The animals with the larger loops absorbed the greater amount of pure sugar in one hour's time and also showed a greater variation in quantity of glucose absorbed under the influence of Ca and K than did the animals with the smaller loops. There seems to be a direct relationship between the surface area of the loop and the amount of variation of glucose absorption occasioned by the addition of potassium chloride or calcium chloride. However, the per cent of increase or decrease in each animal is quite constant.

TABLE 1

Grams of glucose absorbed in one hour from jejunal loops following injection of 10 cc. of a 10 per cent solution together with the variation produced by the addition of a 0.1 per cent solution of K or Ca when allowed to act for the same length of time

DOG 1			DOG 2			DOG 3			DOG 4			DOG 5			DOG 6			DOG 7		
Control	K	Ca	N	K	Ca	N	K	Ca	N	K	Ca	N	K	Ca	N	K	Ca	N	K	Ca
0.426	0.427		0.615	0.698	0.512	0.422		0.411	0.243	0.306	0.200	0.368	0.374	0.341	0.515	0.552	0.462	0.333	0.372	0.313
0.406	0.479		0.605	0.656	0.546	0.443		0.402	0.242	0.310	0.213	0.360	0.377	0.346	0.497	0.561	0.471	0.333	0.367	0.317
0.450	0.482		0.620	0.660	0.576	0.446		0.415	0.244	0.290	0.201	0.359	0.273	0.339	0.517	0.549	0.445	0.319	0.361	0.317
0.449	0.478		0.628	0.654	0.574	0.439		0.398	0.240	0.300	0.210	0.371		0.354		0.556	0.450	0.361		0.311
				0.664	0.568			0.410	0.246	0.270	0.206									
					0.573					0.298										
					0.578															
0.431	0.478		0.611	0.666	0.561	0.437		0.407	0.243	0.295	0.206	0.364	0.374	0.345	0.509	0.555	0.457	0.337	0.366	0.315

In the second series carried out with the closed loops the concentrations of calcium and potassium salts were altered. As in the previous experiment 10 cc. of a 10 per cent glucose solution were used to which the electrolyte was added before injecting it into the loop. This was allowed to remain in the loop for one hour. The remaining material was aspirated and the loop washed with normal saline. The amount of sugar absorbed was then calculated and the effect of the different concentrations of the electrolytes on the absorption rate determined. These concentrations varied from 0.03 per cent to 0.15 per cent for each salt. The results are

TABLE 2

The absorption of glucose as affected by varying the concentration of the added electrolyte (the per cent concentration indicated at top of chart)

Ten cubic centimeters of a 10 per cent solution of glucose were injected and left for one hour. The amount of absorption is indicated in grams.

	CONTROL	0.03 K	0.05 K	0.08 K	0.1 K	0.15 K	0.03 Ca	0.05 Ca	0.08 Ca	0.1 Ca	0.15 Ca
Dog 8	0.471	0.481	0.472	0.514	0.526	0.541	0.438	0.451	0.445	0.444	
	0.462	0.487	0.520	0.502	0.503	0.518	0.445	0.449	0.458	0.439	
	0.472		0.506	0.539	0.507		0.464			0.446	
	0.468	0.484	0.499	0.518	0.512	0.530	0.456	0.450	0.451	0.442	
Dog 9	0.288	0.279	0.309	0.316	0.314	0.312	0.274	0.292	0.261	0.259	0.253
	0.276	0.291	0.294	0.321	0.322	0.319	0.282	0.266	0.267	0.273	0.244
	0.302		0.279	0.297	0.300		0.280	0.274		0.250	0.267
	0.289	0.294	0.291	0.312	0.312	0.315	0.279	0.278	0.264	0.261	0.251
Dog 10	0.489	0.492			0.533		0.476		0.450	0.458	
	0.474	0.521			0.514		0.468		0.462	0.448	
	0.480	0.488					0.479			0.444	
	0.481	0.502			0.524		0.471		0.456	0.450	

given in table 2. Again K is shown to cause an increase in absorption in all concentrations used, and the Ca salt to cause a decrease in each instance. The potassium caused a gradual increase in absorption as the concentration of the salt was increased up to 0.08 per cent. Greater concentrations caused no greater rate of absorption. On the other hand, the decrease in absorption produced by the calcium salt is definitely greater as the concentration of the electrolyte is increased up to 0.15 per cent. Concentrations above 0.15 per cent were not used.

Normal rats that had been kept on a uniform diet consisting of the

Steenbock stock ration supplemented with whole milk were used to check the finding on chronic closed loops in dogs. The salts were used in 0.25 and 0.5 per cent concentrations in the 50 per cent dextrose solution. When 0.25 per cent $CaCl_2$ was used the average absorption was 45.3 per cent (min. 36.8 per cent, max. 54.4, 13 rats). The addition of 0.25 per cent KCl to the glucose gave an average absorption of 58.4 per cent (min. 50.3 per cent, max. 68.3, 15 rats). Six rats given 0.5 per cent $CaCl_2$ showed an average absorption of 43.9 per cent while six given a like concentration of KCl with the glucose averaged 60.8 per cent absorption.

These observations seemed to leave no doubt as to the influence of the two salts under the conditions described. The findings conform to those of Gellhorn and Skupa (1933) working on frogs. Magee and Sen (1932) found that 0.32 per cent $CaCl_2$ depressed absorption in normal rats but when used in 0.02 per cent concentration the absorption rate was increased. While the lowest concentration of $CaCl_2$ used in our closed loop dogs was 0.03 per cent, this amount gave a distinct depression in almost every instance. There is an advantage in using the chronic closed loop in that the experiment can be repeated a number of times on the same animal. Magee and Sen obtained an increase in absorption of glucose in rats and rabbits, where the serum Ca had been lowered by parathyroidectomy, by adding Ca to the glucose. This work was not confirmed by McDougall (1935). By contrasting the two electrolytes a more definite impression as to their action on absorption has been obtained. If these results may be explained by alteration of cell permeability by the two electrolytes our work conforms to the well established effects of K and Ca ions on cell permeability.

SUMMARY AND CONCLUSIONS

KCl when added to a 10 per cent glucose solution in concentrations from 0.03 per cent to 0.15 per cent increases the rate of absorption of the sugar solution from chronic closed intestinal loops in dogs; in similar concentrations $CaCl_2$ decreases the rate of absorption below normal. The increase produced by a 0.1 per cent solution of KCl is approximately equal to the decrease brought about by a similar concentration of $CaCl_2$. The favorable action of KCl reaches a maximum at about 0.08 per cent under these conditions. The retarding action of $CaCl_2$ increases steadily up to 0.15 per cent which was the highest concentration used.

Normal rats given 2 cc. of a 50 per cent glucose solution to which KCl was added (0.25 and 0.5 per cent), absorbed more of the sugar than rats given a similar quantity of glucose to which like amounts of $CaCl_2$ were added.

From these experiments the conclusion is drawn that K ions favor absorption of glucose from the intestine while Ca ions retard absorption.

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ENTEROCRININ, A HORMONE WHICH EXCITES THE GLANDS OF THE SMALL INTESTINE

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In 1935 Nasset, Pierce and Murlin demonstrated a humoral factor in the control of intestinal secretion. Later it was shown that a crude extract, prepared from the intestines of dogs and swine, when injected intravenously excited the glands of the jejunum (Nasset and Pierce, 1935). The secretagogue activity of the extracts was not due to their vasodilatin content as was shown by the destruction of the former but not the latter in boiling alkali.

This paper deals with the distribution, among various tissues of several species, and the preparation of a hormone which is a potent excitant for the glands of the small intestine. The name *enterocrinin* (intestinal secretagogue) is proposed for this hormone.[1] Preliminary reports of this work have appeared (Nasset, 1936; 1937).

METHODS. *Chemical.* Crude preparations were made by 48 hour extraction of fresh tissue in the cold with 3 liters per kilo of 0.03 to 0.05 normal HCl in 85 to 87 per cent ethyl alcohol. After decantation the alcohol was distilled off at low temperature (water bath at 40 to 50°C.) and pressure and the aqueous residue finally made up to one-sixth of the original volume with water. The pH was adjusted to 5.8 to 6.0 and the flocculent precipitate removed and discarded. The clear but colored extract thus obtained was the preparation used in the determination of the distribution of enterocrinin.

A much more refined product in powder form was prepared as follows: The crude extract (from the small gut of swine) described above, was saturated with NaCl without adjustment of pH and the salt precipitate removed by filtration at the pump. (The precipitate may be washed once with saturated NaCl solution to remove adsorbed vasodilatin but this procedure results in a considerable loss of hormone.) The precipitate, after drying in air was shaken twice for one or two hours in glacial acetic acid (10 cc. per gram). The enterocrinin, together with inactive material and some vasodilatin, was precipitated from acetic acid solution by the addition of $2\frac{1}{2}$ to 4 volumes of ethyl ether. This precipitate, after washing

[1] The writer gratefully acknowledges the guidance of Professor Ryland M. Kendrick of the Greek Department in the final selection of the name *enterocrinin*.

with ether and drying, was dissolved in a small volume of acetic acid and re-precipitated with ether. A white, amorphous, water soluble powder was obtained which was effective in doses of 0.5 to 1.0 mgm. per kilo of body weight. Based on the total organic solids present this represents a 10- to 5-fold increase in potency as compared with the crude extracts.

A further purification was effected by precipitation with picric acid. To a water solution (25 mgm. per cc.) of the final product described in the previous paragraph was added an equal volume of a saturated aqueous picric acid solution. After standing in the cold over night the precipitate was centrifuged off, washed once with half saturated picric acid and dried in a vacuum desiccator over H_2SO_4. The enterocrinin picrate was triturated and shaken twice for 2 to 3 hours in a volume of normal HCl equal to the volume of the original aqueous solution. The insoluble residue was removed by centrifugation and discarded. The supernatant fluid was freed of picric acid by shaking with several portions of ether, neutralized with strong NaOH and taken to dryness at about 60°C. The enterocrinin was extracted from the residue with glacial acetic acid, leaving behind the excess NaCl, and precipitated as usual by the addition of ether. This process further increased the potency of the product so that doses of 0.2 to 0.3 mgm. per kilo were effective.

The yields thus far have been disappointingly small, being of the order of 25 mgm. from 1 kilo of fresh small intestine. Large losses occur in the salting out of the crude extract. The filtrate from this step always contains a great deal of enterocrinin as well as vasodilatin and no method has yet been found which will simultaneously increase the yield of hormone and separate it from vasodilatin.

Assay. The enterocrinin content of all crude extracts and progress in isolation of the hormone were determined by the use of the acute method described in previous papers, using dogs as experimental animals (Nasset and Parry, 1934; Nasset and Pierce, 1935). This method has proved to be rapid, reliable and capable of showing small changes in secretory activity not detectable with fistulous animals. However, the final trial must be made with the latter type of test animal and therefore there are included two assays made with a dog bearing a "second stage" intestinal transplant in the belly wall. The technic of the preparation and management of this type of fistula was described in earlier papers on this subject.

Results. In the small intestine of the anesthetized dog fluid exchange in the intestine is directly related to the intraintestinal pressure (Wells, 1931; Nasset and Parry, 1934). If appropriate provision be made for varying the latter, the apparent basal rate of secretion may also be varied at will. In the assay of enterocrinin the basal rate was always set at approximately one drop or less per minute. An increase of one drop per minute over the basal rate for the five minutes following intravenous

injection of any substance was taken arbitrarily to indicate the presence of a significant amount of intestinal secretagogue.

Table 1 epitomizes the results obtained with crude extracts prepared as described in paragraph 1 under "Methods." Each figure is the average of two or more experiments. It is evident that enterocrinin is found only in the small intestine and colon with the exception that its presence in the rabbit GI tract appears rather doubtful. This seems to corroborate the observation of Florey and Harding (1935a) that a humoral control of intestinal secretion in the rabbit is not demonstrable. Furthermore, it appears questionable whether the human or monkey colon contains enterocrinin. Nearly all of the extracts represented in table 1 contained vasodilatins but the response of the intestinal glands apparently is not directly related to changes in blood pressure.

Since many of the extracts necessarily contained secretin, the major pancreatic duct was cannulated in nearly all of the acute experiments.

TABLE 1

Species and tissue distribution of enterocrinin

(Figures indicate drops of intestinal secretion per minute. Control values in parentheses. Acute method)

	STOMACH	SMALL GUT	COLON	LIVER	PANCREAS	KIDNEY	SKELETAL MUSCLE	CECUM
Dog	(0.0) 0.0	(0.7) 5.4	(0.5) 4.2	(0.6) 0.8	(0.8) 1.0	(0.6) 1.2	(0.5) 0.8	
Hog		(0.4) 4.2	(0.4) 4.6					
Cat	(0.6) 0.7	(0.3) 1.6	(0.5) 4.3	(0.7) 0.4	(0.2) 0.0	(0.6) 0.3	(0.2) 0.3	
Human	(0.4) 0.2	(0.4) 1.5	(0.6) 1.3					
Monkey		(0.4) 2.2	(0.3) 0.6					
Rabbit	(0.0) 0.6	(0.6) 1.0	(1.0) 1.4					(0.7) 1.2
Cow		(1.2) 5.9						

It soon became evident that secretin was not the substance which excited the intestinal glands. In figure 1a is shown the effect of injecting 5 cc. of a crude cat colon extract. The small intestine responded with about 4 drops per minute whereas the pancreas remained inactive. This also was the first instance in which a crude extract was vasodilatin-free. Figure 1b shows the presence of enterocrinin and the total absence of secretin in a dog colon extract. The next three records show the activity of extracts made from different parts of the GI tract of a single cat. The stomach extract (fig. 1c) contains no enterocrinin and some secretin, the small intestine contains both in considerable amount (fig. 1d), and the colon is again shown to contain only enterocrinin (fig. 1e). The blood pressure changes were almost identical for the three extracts. The last four records in figure 1 were obtained consecutively on the same test animal.

Figure 2 shows five records obtained successively on one animal. Injection of a crude hog gut extract (fig. 2a) is followed by secretion in both

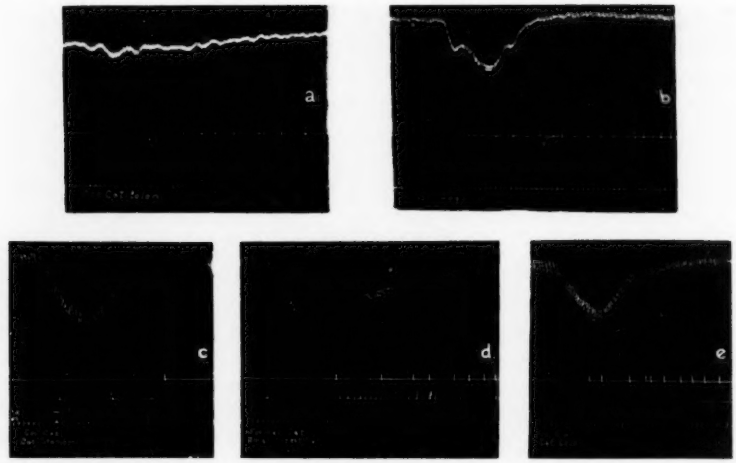

Fig. 1a. Effect of injecting 5 cc. crude cat colon extract.

Fig. 1b. Presence of enterocrinin and total absence of secretin in dog colon extract.

Fig. 1c, 1d, 1e. Activity of extracts made from different parts of G.I. tract of a single cat.

Fig. 1c. No enterocrinin, some secretin; 1d both enterocrinin and secretin, 1e only enterocrinin. First line below blood pressure is intestinal secretion; the second is pancreatic secretion in all figures.

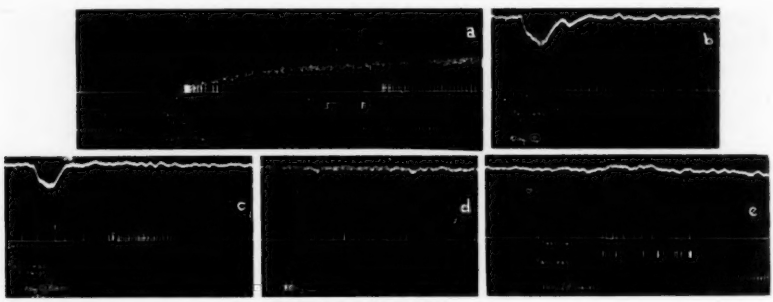

Fig. 2a. Effect of injection of crude hog gut extract.

Fig. 2b. Effect of acetic acid-ether precipitate from crude extract.

Fig. 2c. Showing 3-fold increase in potency and decrease in vaso-dilatin content by precipitation with picric acid.

Fig. 2d. Effect of 0.4 mgm. per kilo of vaso-dilatin-free enterocrinin.

Fig. 2e. Control injection of secretin.

intestine and pancreas, and a profound fall in blood pressure. A dose of 0.4 mgm. per kilo of an acetic acid-ether precipitate from the same crude extract (described in the second paragraph under Methods) provoked a 3.4 drop per minute increase in intestinal secretion with no effect upon

the pancreas (fig. 2b). The potency of this product was increased three-fold and its vasodilatin content decreased by precipitation with picric acid (fig. 2c). A dose of 0.4 mgm. per kilo of vasodilatin-free enterocrinin gave the response reproduced in figure 2d. It should be noted that the three preparations just enumerated did not affect the pancreas. To check the excitability of the pancreas a dose of secretin was injected (fig. 2e). It is common experience to find some enterocrinin in most preparations of secretin and it is probably this coincidence that has led to the belief, often expressed in text books of physiology, that secretin controls the secretion of the small intestine as well as that of the pancreas. The

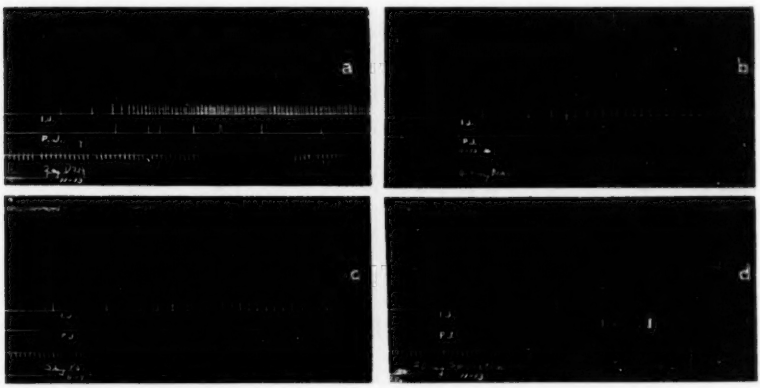

Fig. 3. Results from injection of best lots of enterocrinin.

Fig. 3a. Intestinal secretion increased 19 drops per minute by dose of 70 γ per kilo.

Fig. 3b. One-fifth response in intestine from $\frac{1}{4}$ above dose. No pancreatic secretion.

Fig. 3c. Effect of 0.17 mgm. per kilo picric acid precipitate from hog gut extract. Vasodilatin and secretin free.

Fig. 3d. Control injection of secretion.

proof of the existence of secretin-free enterocrinin is sufficient to refute this idea.

Figure 3 shows the results obtained from injection of two of the best lots of enterocrinin thus far prepared. The first preparation was made from dog's small intestine and a dose of 70γ per kilo increased the intestinal secretion 19 drops (1 cc.) per minute (fig. 3a) with a slight effect on the pancreas. One-fourth of this dose (fig. 3b) gave about one-fifth of the response in the intestine and contained less than a threshold dose of secretin. This indicates a threshold dose of about 6 to 8γ per kilo for this lot of enterocrinin. Since there was doubtless considerable inert material even in this preparation, the indications are that this hormone is effective in rather small doses. Figure 3c shows the effect of 0.17 mgm.

per kilo of a picric acid precipitate from hog gut extract. It is vasodilatin- and secretin-free. The usual control injection of secretin is shown in figure 3d.

In an earlier paper (Nasset and Pierce, 1935) it was demonstrated that in acute experiments the intravenous administration of crude extracts of hog intestine increased the secretion of the enzymes as well as the fluid of succus entericus. It remained now to put the purified enterocrinin to the crucial test of administration to fistula-bearing animals. Several such tests were made of which two are represented in figure 4. This dog had a double fistula of upper jejunum in the belly wall completely isolated from its original mesenteric connections. The enterocrinin injected was from the same lot as that used in a previous acute experiment (fig. 2c). It is evident that the amount of amylase secreted, as well as the volume

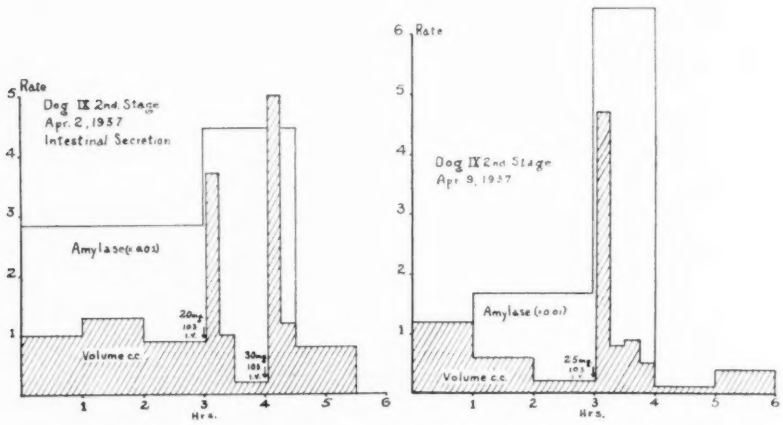

Fig. 4. Administration of purified enterocrinin to a fistula dog

of juice, was quite appreciably increased. Although only amylase activity was determined, it is known from other experiments that at least four enzymes of succus entericus vary in a parallel fashion (Bourns, Nasset and Hettig, 1936).

Discussion. Ågren (1934) reported that his crystalline secretin increased the intestinal secretion in the cat but he based his conclusions on a few results obtained by a method of doubtful validity. The concentration of bicarbonate in succuss entericus varies considerably and, therefore, cannot be used as a reliable index of secretion.

Florey and Harding (1935 b) stated that secretin is the hormone which excites Brunner's glands. The fluid obtained from a duodenal fistula, such as used by these authors, is probably only partially secreted by Brunner's glands and, since the secretin administered was not chemically

pure, it is quite likely that it may have contained enterocrinin. The confusion concerning the exact functions of these two hormones in the physiology of the intestine can be eliminated only when both of them are obtained in the chemically pure form.

On the basis of experimental evidence presented in this paper it is justifiable to conclude that enterocrinin is an intestinal hormone, not previously described, which plays an important rôle in the secretion of succus entericus. Its presence in the colon suggests a possible effect on the functioning of this organ but no work has been done along this line. However, a few preliminary experiments with the acute method showed that the secretion of the ileum is increased by administration of this hormone. It was thought also that it might exert some influence on the motility of the gut but exploratory observations with the balloon technic failed to support this idea.

As yet not much is known concerning the chemical nature of enterocrinin. Aqueous solutions of the best preparations gave positive Millon's, biuret, xanthoproteic, and tryptophane tests, and a negative Molisch test. It is insoluble in fat solvents. In solution at pH 5 to 6 it migrates to the cathode.

SUMMARY

1. The name *enterocrinin* is proposed for a hormone which has the property of exciting the glands of the small intestine.

2. This hormone is obtainable from the small and large intestines of several species of animals. The secretagogue activity of crude extracts of these organs is not directly related to blood pressure changes because vasodilatin-free enterocrinin has been prepared.

3. Enterocrinin does not excite the pancreas; hence it is separate and distinct from secretin. It augments the secretion of enzymes as well as fluid, a property usually not ascribed to secretin.

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THE RELATIONSHIP OF CALCIUM ION CONCENTRATION TO THE COAGULATION OF CITRATED PLASMA[1]

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The rôle of calcium in the coagulation of blood has been reviewed by Howell (1, 2) Wöhlisch (3) and more recently by Ferguson (4). While, as originally suggested by Sabbatani (5) it appears that calcium ions are essential for coagulation, and that partial suppression of the calcium ion concentration, by means of citrate, for example, suffices to prevent or delay coagulation, there are no quantitative data in the literature bearing directly upon this relationship. This study was undertaken in order to determine the minimum concentration of calcium ions at which coagulation occurs, and the relationship between calcium ion concentration and coagulation time. The effects of variations in hydrogen ion concentration were also considered.

METHODS. The observations reported depended upon observation of the time required for coagulation of recalcified citrated diluted plasma, and upon calculation of the Ca^{++} concentration from the total citrate and total calcium concentrations present. The total protein content of the plasma was also determined, but with the concentrations of calcium, citrate, and protein present in the dilutions of plasma used the amount of calcium bound to protein was negligible, and was disregarded (6).

Blood (human or dog) was drawn into one quarter of its volume of 2 per cent sodium citrate, centrifuged, and the plasma separated. Total calcium was determined by the method of Clark and Collip (7) and total citrate by a gasometric method essentially as described by Kuyper (8). The protein was precipitated by the Folin-Wu reagent, and citrate analyses were performed upon the supernatant fluid. Blank readings were obtained on non-citrated serum from the same animals, and these values being small and relatively constant, an average figure was used for correction of all analyses on citrated plasma.

Ca^{++} concentrations were calculated from the total calcium concentration (which was the sum of the calcium present in the plasma and that added in recalcification) and the total citrate concentration. Calculations of Ca^{++} concentrations at pH 7.0 or above were made with a nomogram

[1] This work was aided by a grant from the Josiah Macy, Jr. Foundation.

constructed from the dissociation constant of calcium citrate[2] as found by Hastings et al. (9). At pH below 7.0 the dissociation constant for CaHCit as determined by Muus and Lebel (10), was also taken into account, and calculations were made by means of a grid nomogram from which the Ca^{++} concentration could be read directly from values for total calcium, total citrate, and pH. On account of considerations of space this nomogram is not reproduced but will be supplied by the authors upon request.

The validity of the calculations was tested by citrating and recalcifying serum and comparing the calculated Ca^{++} concentrations with those observed directly by the frog-heart method. Satisfactory agreement was obtained. The validity of the calculations is further supported by the confirmation by Muus and Lebel (10) of the dissociation constant of calcium citrate as found by the frog-heart method and as used in these calculations.

Recalcification and observation of coagulation time were performed in Wassermann tubes. A series of tubes was prepared containing graded amounts of a standard M/10 solution of $CaCl_2$ and a constant amount of buffer solution (see below) and the volume in each tube was brought to a constant amount, usually 1.5 cc., by the addition of 0.9 per cent NaCl solution. Citrated plasma, 0.5 cc., was then added to each tube, and the contents were mixed by inversion. The coagulation time was read as the time required until the tubes could be inverted without escape of the contents. In order to test the possible effect of dilution upon coagulation time dilutions of citrated plasma from 1:2 to 1:6 were compared. Such variations as were observed at constant Ca^{++} concentration were small and irregular, and within the limits of experimental error.

In some of the experiments at constant pH a uniform amount (0.5 to 1.0 cc. for 2.0 cc. total volume) of M/10 sodium veronal[3] buffer solution at pH 7.44, determined electrometrically, was added to each tube. By comparing buffered with unbuffered plasma it was found that the veronal added had no effect upon the Ca^{++} concentration required for clotting or upon the coagulation time.

Electrometric pH determinations were made on control tubes, set up as in the case of the experimental tubes, except that $CaCl_2$ was omitted in order to avoid clotting.

Effect of varying calcium ion concentration at constant pH. The minimum time required for coagulation, at optimum Ca^{++} concentration, varied considerably in different experiments, but averaged 10.2 minutes for human plasma and 5.7 minutes for dog plasma (table 1). In individual experiments the observed coagulation time decreased relatively little as the Ca^{++} concentration was increased above 1.25 mM per liter. As the Ca^{++} con-

[2] $pK_{CaCit^-} = 3.22$; $pK_{CaHCit} = 2.3$.

[3] We are indebted to Dr. E. S. G. Barron for suggesting the use of veronal as a buffer, and for the electrometric determinations of pH.

centration was decreased below this level the coagulation time increased more rapidly until a point was reached at which coagulation would no longer occur. The minimum Ca^{++} concentration at which coagulation occurred averaged 0.35± standard deviation 0.04 millimol per liter for

TABLE 1

Constants for human and dog plasma, as calculated from observations at pH 7.0–8.0

EXPERIMENT NUMBER	BUFFER	DILUTION OF PLASMA	OBSERVED				CALCULATED		
			pH	Ca^{++}		Minimum coagulation time	m	n	K
				Minimum effective	Maximum ineffective in 100 minutes				
Human plasma									
				mM/L.	*mM/L.*	*minutes*			
28	Veronal	1–1		0.37	0.29	11.0	0.30	10.0	3.68
		1–4		0.34	0.26	9.5	0.28	8.5	3.86
		1–6		0.30	0.24	12.5	0.26	10.0	3.94
30	Veronal	1–4	[7.0]*	0.40	0.30	13.0	0.34	13.0	5.96
		1–4	[7.4]	0.40	0.30	11.0	0.34	9.8	5.01
		1–4	[7.8]	0.40	0.30	12.0	0.33	11.2	3.55
31	Veronal	1–4	7.45	0.28	0.21	9.5	0.22	8.5	3.47
		1–4	7.83	0.35	0.28	8.5	0.26	8.5	3.87
32		1–4	7.60	0.29	0.22	9.0	0.21	9.0	4.44
		1–4	7.82	0.37	0.29	6.5	0.28	6.5	2.98
		1–4	7.94	0.37	0.29	7.0	0.21	7.0	4.70
43		1–4		0.32	0.26	13.5	0.27	12.5	3.93
Mean				0.35	0.27	10.2	0.28	9.6	4.12
Standard deviation				±0.04		±2.2	±0.04	±1.9	±0.77
Dog plasma									
37		1–4		0.23	0.18	4.0	0.19	3.0	3.27
38		1–4		0.23	0.18	7.0	0.16	6.0	3.93
39		1–4		0.28	0.22	6.0	0.22	4.5	2.42
40		1–4		0.24	0.18	5.0	0.18	4.0	1.59
42		1–4		0.24	0.18	6.5	0.15	5.5	3.20
Mean				0.24	0.19	5.7	0.18	4.6	2.88
Standard deviation				±0.02		±1.1	±0.02	±1.1	±0.81

* The brackets [ ] indicate that the pH was estimated.

human plasma, and 0.24± standard deviation 0.02 millimol per liter for dog plasma. Tubes requiring more than 100 minutes to clot were not included in these observations because it was felt that after longer standing other variables might manifest themselves.

When coagulation time was plotted against Ca^{++} concentration the curve took the form of a rectangular hyperbola (fig. 1). The data from each sample of citrated plasma could be fitted with a considerable degree of accuracy to the empirical equation

$$t = \frac{K}{Ca^{++} - m} + n \tag{1}$$

in which t = time in minutes, Ca^{++} = calcium ion concentration in millimols per liter, K is a constant, and m and n are constants representing the asymptotes of the curve.

When different plasmas were compared, the values for K, m and n as calculated from the observations varied considerably (table 1). Consequently, when data from different experiments were plotted together

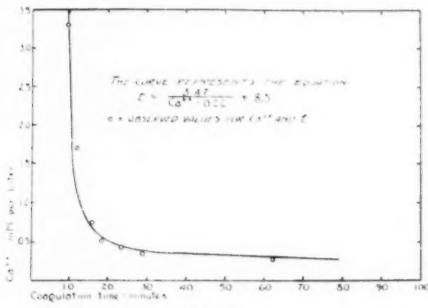

Fig. 1

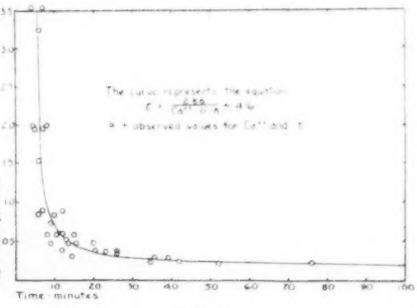

Fig. 2

Fig. 1. Relation of observed values to curve calculated from constants for human plasma. Expt. BC., no. 31, series A. pH, 7.45. Each tube contained 1.0 cc. veronal buffer and 0.50 cc. citrated plasma.

TUBE	100 mM $CaCl_2$	154 mM NaCl	OBSERVED		CALCULATED
			t	Ca^{++}	t
	cc.	*cc.*	*minutes*	*mM/L.*	*minutes*
1	0.03	0.47	No clot	0.17	
2	0.04	0.46	No clot	0.21	
3	0.05	0.45	62.0	0.28	64.3
4	0.06	0.44	29.0	0.35	35.2
5	0.07	0.43	23.5	0.43	25.0
6	0.08	0.42	18.5	0.52	19.7
7	0.10	0.40	16.0	0.75	15.1
8	0.15	0.35	12.0	1.70	10.8
9	0.20	0.30	9.5	3.30	9.6
10	0.25	0.25	9.5	5.20	9.2

Fig. 2. Comparison of curve calculated from mean constants with observed values—dog plasma.

the agreement between the points and a curve constructed from average values of the constants was less striking. (Figs. 2 and 3.) The differences between human and dog plasma, however, appeared to be definite and constant.

Significance may be assigned to the constants m and n in equation (1) as follows: m obviously represents the limiting concentration of Ca^{++} and n represents the limiting value for t. In physiological terms m is the value of Ca^{++} concentration for coagulation at infinite time, and n is theoretically the value of t for coagulation at infinite Ca^{++} concentration. With respect to the latter it should be pointed out that the relationship expressed in equation (1) no longer obtains at very high Ca^{++} concentrations, since under such conditions clotting may be greatly slowed or actually prevented (5). The calculated values for m in table 1, which are

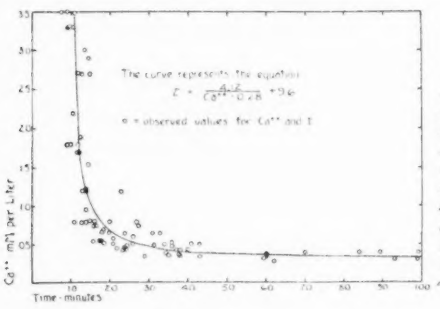

Fig. 3

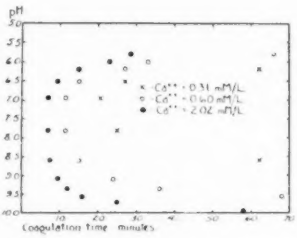

Fig. 4

Fig. 3. Comparison of curve calculated from mean constants with observed values—human plasma.

Fig. 4. Relation of coagulation time to pH at constant Ca^{++} concentration—dog plasma.

lower than the minimal values of Ca^{++} concentration at which coagulation was actually *observed* to occur, are extrapolations of the experimental data to the conditions at infinite time.

Effect of varying pH. Only a few experiments were performed in which pH was varied in the presence of constant and varying Ca^{++} concentrations. Data from such an experiment are plotted in figure 4. In general terms it may be said that there is little change in the coagulation time between pH 7.0 and pH 8.0, and that the optimum pH (at which clotting occurs in the shortest time at constant Ca^{++} concentration) lies between these two values. As the pH shifts further toward the acid or alkaline side the coagulation time increases, slowly at first, then more rapidly. The greater the Ca^{++} concentration the less is the effect of variation in pH, the pH effect being slight at optimal Ca^{++} concentrations and most marked

at Ca^{++} concentrations just above the minimal effective concentration. From our data it would appear that a shift in pH is accompanied by changes in all three of the constants, K, m and n, of equation 1. At high or low pH the minimal coagulation time is increased, as well as the minimal Ca^{++} concentration necessary for clotting. Further analysis of these changes is not possible from the data at hand.

Discussion. The experiments reported throw no new light upon the mechanism of the coagulation of blood. They do provide quantitative information as to the minimal Ca^{++} concentration necessary for the coagulation of blood, and as to the effect of varying Ca^{++} concentrations upon coagulation time, and present, in qualitative form, some indication of the effects of pH upon both of these factors. It is of interest that the minimal Ca^{++} concentration effective in promoting the coagulation of blood is, in the case of the dog at least, below the minimal Ca^{++} concentration reported as being compatible with the life of the animal. (McLean, Barnes, and Hastings (11): 0.35 millimol per liter.) It is also of interest that increase in calcium ion concentration above that normally present in the plasma (1.25 millimols per liter) exerts relatively little effect on coagulation time, and that changes in pH compatible with life do not appreciably alter the coagulation time.

SUMMARY AND CONCLUSIONS

1. The minimal Ca^{++} concentration at which coagulation of diluted citrated plasma at pH 7.0 to 8.0 was observed to occur averaged 0.35 $\pm$ standard deviation 0.04 millimol per liter for human plasma and 0.24 $\pm$ standard deviation 0.02 millimol for dog plasma. When extrapolated to infinite time these values become 0.28 millimol per liter for human plasma and 0.18 millimol per liter for dog plasma. The differences between human and dog plasma appear to be constant and real.

2. The minimal coagulation time, for both human and dog plasma at pH 7.0 to 8.0 is approached above Ca^{++} concentrations of about 1.25 millimols per liter. The minimal coagulation time averaged 10.2 minutes for human plasma and 5.7 minutes for dog plasma.

3. For a given plasma at constant pH the relationship between Ca^{++} concentration and coagulation time can be expressed by the empirical equation

$$t = \frac{K}{Ca^{++} - m} + n$$

in which t = time, Ca^{++} = calcium ion concentration in millimols per liter, K is a constant, m is a constant expressing Ca^{++} concentration for clotting at infinite time, and n is a constant expressing coagulation time

at infinite Ca^{++} concentration. The values of the constants K, m and n vary for different samples of plasma.

4. At any given Ca^{++} concentration the minimum coagulation time is observed when the pH is between 7.0 and 8.0 A shift of pH beyond these limits in either direction results in an increase in coagulation time, as well as in the minimum Ca^{++} concentration required for clotting. The effect of change in pH increases with decreasing Ca^{++} concentration.

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THE EFFECT OF BODY TEMPERATURE ON REACTION TIME

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Diurnal variability in mental, motor and sensory performance has been studied by many investigators. The variability in conclusions is as numerous. Curves have been described with *a*, continuous rise; *b*, continuous fall; *c*, morning rise, afternoon fall; *d*, morning fall, afternoon rise; *e*, no curve whatsoever, and various curves with several notches. In 1906 Marsh (1) published an extensive study in which he reported instances of each of these types of curves. More recently, Freeman and Howland (2), in a review of the literature, list many examples of each type of curve.

None of these many workers, however, made any observations of body temperature at the time of testing the subjects prior to Kleitman (3) who published diurnal curves with morning rises and afternoon falls for speed and accuracy of a variety of tasks, such as dealing cards, code transcription, multiplication, mirror drawing, as well as the curve for temperature observed at the time of the tests. Increase in temperature appeared to improve performance markedly.

The present study was designed to investigate the possible diurnal character of rather simple performances, likewise in relation to body temperature. It was also desired to compare the influence of temperature fluctuations on simple tasks with their influence on more complicated tasks. The performances selected for this purpose were purely sensory-motor: reaction to light and reaction to sound, and sensory-mental-motor: choice reactions to light and sound.

METHODS. The time required to react to visual and auditory stimuli was studied. Five subjects, male graduate students and an instructor, participated, each acting alternately as subject and observer. The tests were made at various times of the day: at 9 a.m., 11 a.m., 1 p.m., 3 p.m., 5 p.m., 7 p.m. and 9 p.m., except for one subject (N.B.) who was tested at 7 a.m., 9 a.m., 11:30 a.m., 6 p.m. and 11 p.m. and one subject (N.K.) who was tested at 9 a.m., 11 a.m., 3 p.m., 5 p.m. and 9 p.m. A complete test consisted of twenty trials each for *a*, simple reaction: to white light; *b*, simple reaction: to sound of a telegraph key; *c*, choice reaction: response to

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green light, but not to red; *d*, choice reaction: response to sound of a bell, but not to the telegraph key. The choice auditory reaction was added after the study had been begun and therefore there are fewer figures for that reaction.

TABLE 1

Diurnal variation in reaction time in relation to diurnal variation in oral temperature

SUBJECT	HOUR OF DAY	ORAL TEMPERATURE	AVERAGE REACTION TIME*			
			Simple: visual	Simple: auditory	Choice: visual	Choice: auditory
		°F.	σ	σ	σ	σ
S. T. (I)	9 a.m.	97.94	155.2 (10)	144.9 (10)	258.7 (11)	275.6 (6)
	11 a.m.	98.18	144.6 (13)	136.3 (13)	248.1 (12)	258.5 (6)
	1 p.m.	98.34	139.9 (12)	134.3 (12)	236.3 (11)	249.2 (4)
	3 p.m.	98.60	143.3 (18)	137.0 (18)	234.7 (19)	244.2 (11)
	5 p.m.	98.58	149.6 (9)	141.5 (9)	236.1 (9)	256.5 (4)
	7 p.m.	98.38	150.4 (8)	138.0 (8)	238.8 (8)	247.7 (7)
	9 p.m.	97.96	161.6 (6)	157.3 (6)	254.7 (6)	266.0 (5)
P. F.	9 a.m.	97.81	138.4 (5)	135.2 (5)	277.0 (5)	249.2 (4)
	11 a.m.	98.10	142.2 (17)	134.2 (17)	267.5 (17)	256.6 (11)
	1 p.m.	98.05	140.2 (16)	130.3 (16)	248.6 (16)	235.8 (10)
	3 p.m.	98.23	142.4 (24)	139.0 (24)	250.1 (24)	245.3 (11)
	5 p.m.	97.84	150.4 (8)	136.8 (8)	261.0 (8)	233.2 (5)
	7 p.m.	98.15	151.2 (5)	139.2 (5)	255.8 (5)	246.3 (3)
	9 p.m.	97.60	154.0 (2)	149.5 (2)	276.0 (2)	275.5 (2)
N. K.	9 a.m.	98.18	184.9 (11)	188.0 (11)	298.7 (11)	328.5 (9)
	11 a.m.	98.16	183.9 (9)	178.1 (7)	311.1 (7)	296.2 (5)
	3 p.m.	98.75	181.4 (5)	187.4 (5)	294.4 (5)	286.5 (6)
	5 p.m.	98.50	186.7 (3)	172.7 (3)	326.0 (3)	271.0 (4)
	9 p.m.	97.65	190.5 (2)	183.5 (2)	319.0 (2)	
N. B.	7 a.m.	97.92	223.0 (5)	173.8 (23)	378.4 (23)	
	9 a.m.	98.12	201.2 (5)	169.0 (22)	365.4 (23)	
	11:30 a.m.	98.23		172.7 (15)	362.1 (14)	
	6 p.m.	98.29	188.5 (2)	168.3 (12)	348.8 (12)	
	11 p.m.	97.95	203.5 (3)	168.4 (18)	370.2 (18)	

* Figures in parentheses indicate number of 20-trial periods.

The apparatus used was a Hipp chronoscope measuring reaction time in thousandths of a second (sigmas). A clinical thermometer held in the mouth for five minutes measured the body temperature after each test.

There was for a time a gradual improvement in reaction time scores, but this improvement was not a factor in the influence of body temperature which manifested itself at different levels of achievement.

RESULTS AND DISCUSSION. The results obtained in this study indicate a

diurnal curve showing a progressive decrease in reaction time during the morning and early afternoon and a rise in the late afternoon and evening (table 1). That this variation in reaction time frequently is related to the diurnal temperature curve can be seen in the sample curve for one of the subjects, giving the diurnal temperature and visual choice reaction time (fig. 1). Since a small reaction time signifies good performance, the ability to respond promptly appears to be best in the middle of the day when the temperature is highest, and poorest in the morning and late evening, when temperature is lowest.

TABLE 2

The relationship between oral temperature and the reaction time to light and sound. Subject S. T.

EXPERIMENTAL GROUP NUMBER	NUMBER OF 20-TRIAL PERIODS	STIMULUS	REACTION TIME WITHIN EACH ORAL TEMPERATURE GROUP						
			97.5°F.	97.8°F.	98.0°F.	98.2°F.	98.4°F.	98.6°F.	98.9°F.
			σ	σ	σ	σ	σ	σ	σ
I	76	Simple visual	145.5	138.6	140.6	136.6	131.5	135.9	134.0
II	68	Simple visual	148.5	142.8	147.0	146.4	143.1	131.4	136.3
I, II	144	Simple visual	146.0	140.1	144.1	140.6	137.3	134.0	135.9
I	76	Simple auditory	138.2	129.0	135.3	128.6	125.1	128.3	122.0
II	68	Simple auditory	134.0	138.5	139.9	136.0	134.4	126.8	125.1
I, II	144	Simple auditory	137.5	132.5	137.8	131.6	129.7	127.7	124.5
I	76	Choice visual	295.1	277.0	280.4	281.0	269.3	263.5	262.7
II	68	Choice visual	265.0	255.7	250.0	246.3	236.7	227.4	223.3
I, II	144	Choice visual	289.7	265.5	266.9	268.4	257.8	252.3	232.6

Temperature groups extend for 0.1°F. above and below the value used to identify the group, except for temperature groups 97.5°F. which includes all values up to 97.7°F. and group 98.9°F., which includes all values of 98.7°F. and above.

This subject performed 144 complete tests. The average reaction times to simple visual and auditory and to choice visual stimuli are presented separately for the first 76 tests and for the last 68 tests, then in combined form, as they fall into body temperature groups. Although experimental group II (68 tests) yields generally lower reaction times than group I (76 tests), particularly for the choice visual stimuli, the same tendency for decrease in reaction time with increase in temperature is apparent.

Fairly good relationship between body temperature and reaction time was observed regardless of the time of day, indicating that there is probably no diurnal reaction time curve independent of the temperature. On the contrary, it would appear that it is always dependent upon the body temperature, which, whenever it becomes changed, is accompanied by a change in the opposite direction in reaction time. The relationship between temperature and reaction time is brought out by the data presented in table 2, which were obtained by averaging together the reaction times for tests made when the temperatures were the same, regardless of time of

day. Thus tests for 9 a.m., 11 a.m. and 9 p.m. might all be averaged together if the temperatures at the time of the tests were, respectively, 97.6°, 97.65° and 97.5°. This inverse relationship is true of both simple and choice reactions.

Further analysis of the temperature and reaction time relationship was made by comparing pairs of successive tests made on the same day. The paired tests for each subject were divided into groups on the basis of whether the oral temperature in the second test was higher or lower than in the first. The corresponding changes in reaction time from first test to second were then examined for number of increases, decreases, and no changes within each temperature change division. The evidence indicates an inverse relationship between reaction time and body temperature changes. There were more than three times as many cases of reaction time changes in the opposite direction to that of temperature than there were cases of their changing in the same direction. An illustration of the effect of changing body temperature on reaction time is shown in figure 2.

The above data were based on spontaneous, or naturally-occurring, diurnal changes in temperature. The same influence of temperature on reaction time can be demonstrated by changing the body posture in the manner described by Kleitman and Doktorsky (4). If, after standing for one hour, one assumes a horizontal position, after another hour the oral temperature will have fallen. In 14 experiments on five subjects that procedure resulted in the following changes, with the ranges for the five subjects given in parentheses: oral temperature, -0.64°F. ($-0.45°$ to $-0.90°$); simple visual reaction time, $+30\sigma$ ($+6$ to $+51\sigma$); simple auditory reaction time, $+30\sigma$ (-7 to $+72\sigma$); choice visual reaction time, $+27\sigma$ ($+9$ to $+61\sigma$); choice auditory reaction time, $+30\sigma$ ($+1$ to $+49\sigma$). Several experiments in which the subject first lay down, then stood up, resulted in a rise in temperature and an accompanying decrease in reaction time.

In an attempt to relate quantitatively temperature and reaction time, the data for each subject were graphed, curves fitted by the method of least squares and the Van't Hoff-Arrhenius equation[2] for the influence of temperature on reaction velocities applied. Data on more complex performances studied by Kleitman (3) were treated similarly. One of the curves obtained is reproduced in figure 3, and was drawn to fit the data for visual choice reaction time for subject S. T., experimental group II. The data are taken from table 2. To test the significance of the magnitude of the difference in reaction time in the various temperature groups, the

[2] A convenient form for this equation is: $\ln \frac{k_2}{k_1} = \frac{\mu}{R}\left(\frac{1}{T_1} - \frac{1}{T_2}\right)$. K_1 and K_2 are the velocities of the reaction at absolute centigrade temperatures T_1 and T_2 respectively. R is the gas constant, with the value 2. The solution is for the temperature constant or "characteristic." See Hoagland (5).

standard deviations for the mean values for each of the twenty trials in every test within the various groups were computed. The standard deviations of the differences between the means of two temperature groups were then computed, and the ratio of the difference to the S.D. of the difference determined. This was done for three different stimuli in three different subjects. In all three cases the ratio was better than 3. For the data in figure 3, the difference between the reaction times for the 97.5° and 98.9° groups is 41.7. The S.D. of the difference is 7.8. The differ-

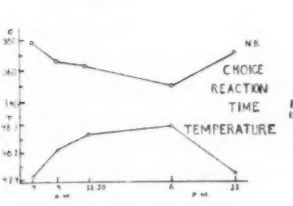

Fig. 1

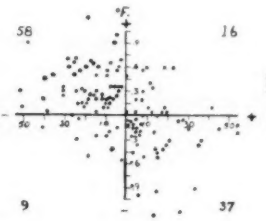

Fig. 2

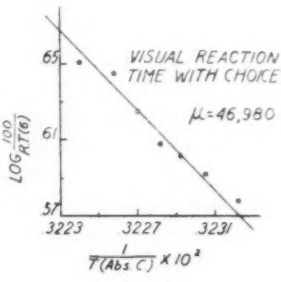

Fig. 3

Fig. 1. Diurnal variation in reaction time to choice visual stimuli for one subject. Reaction time, upper curves, in sigma. Temperature, lower curves, in degrees Fahrenheit.

Fig. 2. The relationship between *changes* in body temperature and *changes* in visual reaction time for subject S. T., based upon 120 *pairs* of observations, usually separated by two or three hours. Body temperature changes are plotted along the ordinates and reaction time changes along the abscissae. In most cases a change in body temperature in one direction is accompanied by a change in reaction time in the opposite direction, 95 cases (58 + 37) showing this relationship, against 25 cases (16 + 9) where the body temperature and reaction time varied in the same sense.

Fig. 3. The relationship between body temperature and reaction time to choice visual stimuli. The curve is fitted by method of least squares. Data from table 2, experimental group II. For purposes of obtaining an expression of the relationship in terms of the Van't Hoff-Arrhenius equation the log of the reciprocal of the reaction time is plotted against the reciprocal of the absolute centigrade temperature. The μ value for the combined groups II and I, a total of 142 tests, is 45,580.

ence is 5.35 as great as the S.D. of the difference, which makes it undoubtedly significant.

Although there was not very good agreement for the μ values obtained for the same task for different subjects, and there were several instances in which the fits were judged poor and doubtful, there did appear a suggestive grouping of the various performances analyzed on the basis of temperature characteristics. The simpler tasks have relatively low temperature coefficients; the more complex tasks are characterized by higher coefficients. The simple reaction times, both to light and to sound, yielded values of

26,000 calories, the choice reaction time had values of about 45,000 calories.[3] Similarly, the data from Kleitman (3) yielded values of 23,000 cal. for sorting of playing cards, but gave higher values of 40,000 to 54,000 calories for the more complex tasks, such as speed of code transcription, mirror drawing, and multiplication.[4] Accuracy of the above tasks showed an improvement many times greater than the velocity of the task, with the same increase in temperature.

The μ values obtained and their grouping according to the complexity of the process whose velocity is being measured gain added interest when compared with those for 360 different biological reactions listed by Crozier (7). In Crozier's paper is published a graph showing multimodal frequency of occurrence of certain μ values. Clear modes are exhibited at 8, 11, 12, 16, 18, 20, 22, 24 and 32 thousand calories. In no case did the comparatively simple biological processes listed by Crozier have values as high as those obtained by us for the more complex mental and motor performances. It would appear that the relatively more potent effect of body temperature on the velocity of the more complicated processes, particularly mental processes, would result in a greater diurnal variability in the rate of these performances than in the variability of the simpler processes.

There is a definite difference in the degree of mental activity required of the subject between the performance designated as simple reaction and that called reaction with choice. In the former alertness is all that is required, but in the latter where the individual is to respond to one type of signal and *not* to another, analysis and judgment are the added requirements. That is reflected in the longer time, roughly one-tenth of a second, needed for reaction with choice. Whereas there may be room for argument concerning the "mental" nature of simple reaction, there can be no question about the process of weighing the evidence which is inherent in making the choice. It is significant that the effect of temperature is more marked with respect to reaction with choice than in simple reaction. Subjectively, the individual tested could allow his mind to wander when his simple reaction time was measured, just getting ready for responding when he received the preliminary warning of the imminence of stimulation, but he had to be "on his toes," figuratively speaking, throughout the period of the testing of his reaction time with choice, if he wished to avoid mistaken

[3] μ values of 26,000 and 46,000 calories correspond, roughly, to Q_{10} values of 5 and 10, respectively.

[4] We were able also to confirm the value of 29,400 cal. obtained by Hoagland and Perkins (6) for the heart rate in man. Their value was computed from data obtained for a range of about 6°F., achieved by use of diathermy to elevate the temperature. Our value of 30,300 cal., computed from Kleitman's data (3) was obtained over a normal range of 0.75°F.

responses and reprimands from the observer. To our knowledge the effect of body temperature on reaction time with choice is the first definite proof of variation in mental work with body temperature.

Assuming that the effect of temperature indicates that we are dealing with a chemical phenomenon, there are two interpretations of the relationship between temperature and reaction time possible: either *a*, mental processes represent chemical reactions in themselves, or *b*, the speed of thinking depends upon the level of metabolic activity of the cells of the cerebral cortex, and by raising the latter through an increase in body temperature we indirectly speed up the thought processes.

SUMMARY

1. There is a diurnal variation in simple and choice reaction times in man, with best performance in the afternoon and poorest early in the morning and late at night.

2. This variation appears to be dependent upon the diurnal body temperature curve being maintained. A spontaneous or induced change in body temperature is reflected in a change in the opposite direction of reaction time.

3. The influence of changes in body temperature is more pronounced upon complex than upon simple processes and performances.

We are indebted to Messrs. O. Berg, N. Brewer and L. Moses for their assistance in this study.

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CHANGES WITH AGE IN THE BASAL METABOLIC RATE IN ADULT MEN[1]

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That alterations in physiological behavior are associated with growth of the body is an obvious biological event. The measurement of such alterations at different periods of life, their increase or decrease, and the rate of these changes—whether rapid or slow—has not been frequently undertaken especially in late adult life.

To add to knowledge of the behavior of the body after age 40 is becoming increasingly important, because of the increase of the population in the older decades and, therefore, the greater number of affections in the aged. For this reason to possess analyses of physiological changes between the 7th and the 9th decades has become desirable, for changes barely detectable in the 5th and 6th have then advanced so as to be clear and measurable. The meaning of the changes, noticed earlier, for the subsequent development of chronic degenerative diseases, can then be appreciated. To be able to make correct descriptions, measurements illustrative of these changes should be collected in an orderly and systematic fashion. In the light of the results, correlations indicative of the interdependences of various organs, as for example of kidneys, heart and lungs, can then be undertaken.

The study now reported has proceeded from this point of view. It is concerned with the period of human life after the fortieth year. The subjects were men, apparently healthy. The functions observed have been 1, the metabolism of the body in terms of the consumption of oxygen; 2, the volume of blood put out by the heart per minute, in association with certain related functions; 3, the blood pressures; 4, the ability of the kidneys to remove the products of nitrogenous metabolism from the blood, and to concentrate the urine; 5, the amount of certain substances in the blood related to the activity of the kidneys and to the state of the arteries; 6, the vital capacity of the lungs; 7, the electrocardiograms; and 8, the state of the heart, great, and long arteries as shown in roentgenograms.

[1] An abstract of these studies was read before the American Society for Clinical Investigation, April 30, 1934.

This paper is no. 17 of the series on Physiological Ontogeny.

There were one hundred men, between ages 40 and 90 years, twenty in each decade, and besides, two men, aged 91 years and one, 101 years. All were physically active; none was an inmate of an institution.

Plan of observations. Each subject was admitted for observation for two days. During the first, the history was taken, the physical examination was made, and the clearance of urea by the kidneys was measured. A rehearsal in the procedures employed in estimating the basal metabolism and the cardiac output was undertaken. On the morning of the second day, these functions and the blood pressures were measured under basal conditions—the subject at rest, after the night's sleep and fourteen hours after the last meal. Blood for analysis of lipid and calcium content was obtained during the fasting state. Arterial blood for estimation of oxygen saturation was collected under oil from the radial artery. The electrocardiograms and the x-ray photographs of the heart, lungs and extremities were taken. The conditions surrounding the men studied in these reports were unusual. They were in hospital and had been there over-night. If the levels of measurement obtained were lower than is usual, it must be due to a state of relaxation of these men. Relaxation is not necessarily the ideal state. It marks a low level of activity, not necessarily the lowest, between which and various degrees of exertion, the consequences of exertion can be observed. To be observed at the low level of relaxation has the value of permitting estimates to be made of effects entailed by work.

The basal metabolic rate. The basal metabolic rate of uniform groups representative as were these of the five decades beginning with the 5th, has not before been studied. After age 70, the number of measurements so far reported is limited (table 1). Even so, as will appear, although the number has been sufficient to learn that the general course of the curve during these decades is downward, it has been insufficient to describe its form in detail decade by decade. The Benedict-Roth universal apparatus was used in most of the cases; the Sanborn apparatus in the rest. The average of two tests was regarded a correct measurement if the two agreed within 6 per cent (1). If the respiratory quotient is regarded as being 0.82, as is permissible, the error of the measurement does not exceed 1 to 2 per cent (2, 3, 4). Throughout adult life, the quotient undergoes no significant change (5, 6).

The calculations of the basal metabolic rate of normal individuals may be presented in two ways. Age, sex, height, enter, of course, as factors into the calculation. The difference in the curves between Harris and Benedict and Du Bois lies in an advantage which Benedict gave to weight—weight having a value, so far as the basal metabolic rate is concerned, greater than the other components in the calculation. This procedure has been usual. Both Harris and Benedict (7) and Du Bois (4, 8) have

TABLE 1

Number of measurements of basal metabolism in normal subjects over 40 years of age previously reported

REPORT NO.	DATE	AUTHOR	SEX	NUMBER OF SUBJECTS BY AGE GROUP														Compare with Report No. 3	Compare with Report No. 4	NOTES
				40 to 44	45 to 49	50 to 54	55 to 59	60 to 64	65 to 69	70 to 74	75 to 79	80 to 84	85 to 89	90 to 94	95 to 99	106	Sum			
1	1899	Magnus-Levy and Falk (23)	♂	1			1	1		2	2						7			
2	1916*	Du Bois (8)	♂	4			1										5			
3	1917*	Aub and Du Bois (5)	♂								3	3					6	+7%		Cf. with Report No. 2
4	1919	Harris and Benedict (7)	♂	5	1		2	3									11	−3 to −4%		
			♀	4	3	6	4	3	1	2							23			
5	1920*	Dreyer (24)																+1 1%		
6	1921*	Blunt and Dye (25)	♀	3														−2 1%	−0 8%	
7	1922	Boothby and Sandiford (26)	♂	3	2	2	4	1									12	+1 0%	+5 7%	
			♀	4	3	7	2	1	1								18	+0 3%	+4 2%	
8	1923*	Krogh (10)																−4 to −6%		
9	1925*	MacLeod and Rose (27)	♀	13														−12 9%	−6 8%	
10	1928*	Benedict (28)	♂	4		1		2					1				8	−4 4%	−1 8%	
			♀	3	2	4	1										10	−7 3%	−4 2%	
11	1929*	Boothby and Sandiford (29)	♂	1822	See note													−1 to −4%		
			♀	5066																
12	1929*	Wolf (30)	♂							2			1				3	−6 8%	+8 3%	
			♀								1						1	+3 2%	+9 0%	
13	1932*	Benedict and Meyer (31)	♀						2	9	4	7	1				23			
14	1932*	Albergo (32)	♂	1				2	8	4	3	4	1				23			
15	1932*	Lucchi (33)	♂			3	1	6	8	9	2	2					31			
			♀					2	2	2	1						7			
16	1934*	Kise and Ochi (34)	♂			5	8	7	8	4	6	4	1	1			44	Slightly lower	Slightly higher	
			♀			2	6	4	12	4	14	7	1				50			

17	1934*	Benedict and Root (35)	♂											1		1	−24 0%	−4 5%	
18	1934*	Matson and Hitchcock (36)	♂						1	5	3	4	1			14	+6 9%	−13 6%	
			♀							1	4	1	1		1	8	−16 4%	−11 6%	
19	1935*	Benedict (21)	♂						2		1	2				5	−10 7%	+3 1%	
			♀					1	3	3	1	2				10	−8 2%	+0 2%	
20	1936*	Boothby, Berkson, and Dunn (20)	♂	61	42	41	26	13								183	?1 3% lower		Cf. with Report No. 11
			♀	64	49	64	41	18	15							251			

* Explanations. (The numbers correspond to the reports.)

2. Curve of metabolism from birth to 80 years, using data on subjects over 40 years of age, based on data in nos. 1 and 2.
3. Subjects were inmates of institutions—not active, and showed stigmata of senility. Curve given from birth to 80 years, using data on subjects over 40 years of age based on data in nos. 1, 2 and 3.
5. Equations for men and women, using data on subjects over 40 years of age, based on data in nos. 1, 2, 3, 4.
6. Included in report on 17 normal women, aged 21 to 44 years.
8. Curves from 15 to 70 years, based on data in nos. 1, 2, 3, 4.
9. Included in report on 92 normal women, aged 20 to 49 years.
10. Records of second normal series from his laboratory; 27 men, aged 21 to 89 years, average 34 years; 33 women, aged 18 to 58 years, average 32 years. Benedict calls attention to distinction between "hospital normal" and physiological standards, the use of the former being justified for clinical purposes only.
11. Curves for men and women from 5 to 70 years, extrapolated to 70 to 80 years. No age distribution reported.
12. Normal subjects.
13. Individual records and curves given in Benedict (1935).
14. Italian subjects. Results given in calories per square meter of body surface per hour with record of body size. Percentage deviations given from Aub-Du Bois table of 40 calories per hour per square meter in young adult men.
15. Italian subjects. Results given in calories per square meter per hour. No records of body size. No significant deviations from prediction values of Harris and Benedict tables.
16. Japanese subjects. Curves for men and women from 50 to 90 years.
17. One normal subject. Includes records of 2 normal men previously reported, one by Benedict, 1928, one by Wolf, 1929.
18. Inmates of institution—not active. Subject of 106 years (birth record not obtained)bed-ridden for 3 years.
19. Subjects observed under home surroundings. In good health and active.
20. Subjects and patients of the Mayo Clinic, selected clinically as being free from metabolic disturbance. Curve and nomogram for men from 6 to 64 years; for women from 6 to 69 years. Boothby, Berkson and Dunn, (1936) include data on 13 men, 60 to 64; and 33 women, 60 to 68 years (their oldest groups).

taken these factors into account. There is a difference between them however in that Du Bois uses the area of the body surface as a proportional, but not causal, measurement of the metabolism, and that Benedict predicts the metabolism from body mass, calculated from an equation based on direct physical measurements and considered to be not only a proportional but also a causal measurement of the metabolism.

Inasmuch as the former uses height and weight in the calculation of surface area and the latter in the calculation of body mass, the practical differences due to the slightly different height-weight factors are small (9, 10). More marked discrepancies in their curves are due to differences in values attributed to age and sex as factors (9).

THE RESULTS. Each of several aspects[2] of the results deserves attention.

a. It is, of course, well known that in a given class of individuals the results of measuring the metabolic rate are scattered over a range sometimes of considerable extent. Of a group selected according to specified criteria, a single figure may not, for this reason, be regarded as descriptive of each member. Actually, no such figure exists. An average may be ascertained and the deviations from that average of all the individuals. The deviations are to be regarded as being equally important as is the average. To discover a scattering, a range of measurements, is in any case the usual experience. That this is a fact deserves and indeed requires recognition. The measurements which have been obtained illustrate this common experience (fig. 1, table 2). In the age group 45 to 49 years, the several deviations from the mean ranged from −15.1 per cent (30.2 calories per square meter per hour in a Y.M.C.A. secretary) to +10.8 per cent (39.2 calories in a Captain in the New York Fire Department). At other age levels similar scattering occurred. In the low range in a younger group, cases are therefore found at the same level as in the high range of an older one. At age 82 an active retired Colonel, U. S. Army, (number LXXXVII) for example, exhibited a rate of 38.2 calories while at age 72, a president of a large New York department store (number LXX), one of 28.1 calories. And yet the rate of the oldest man studied (101 years) was only slightly less than this, though his vigor was on a distinctly lower plane.

b. In a large population exhibiting a wide age span, the metabolic rate varies conceivably with age. There are in fact data, already published, which indicate that this is a fact. A single average figure is, therefore, undoubtedly not descriptive of the metabolic level of the entire population.

It is apparent that the level of the basal rate undergoes a progressive decline (fig. 1) with advancing age and that the decline is described by a

[2] These aspects (a b c d) of this study are still under investigation.

TABLE 2

Measurements of basal metabolism taken of the men examined

GROUP	SUBJECT NO.	AGE†	HEIGHT	BODY WEIGHT (WITHOUT CLOTHING)	BODY SURFACE AREA (DU BOIS WEIGHT-HEIGHT CHART)	OXYGEN CONSUMPTION, 0°C., 760 MM. HG.	METABOLISM				DIFFERENCE FROM CURVES OF	
							Total	Per kgm.	Total	Per sq. meter body surface	Harris Benedict (1919)	Du Bois Boothby Sandiford (1929)
							Calories per 24 hours		Calories per hour			
		yrs.	*cm.*	*kgm.*	*sq. meter*	*cc.*					*per cent*	*per cent*
40–44	I	40	174.0	83.6	1.98	257	1788	21.4	74.5	37.6	−1.5	−1.8
	II	40	164.0	64.2	1.68	228	1579	24.6	65.8	39.2	+5.3	+2.3
	III	41	174.0	66.4	1.79	263	1831	27.6	76.3	42.6	+16.3	+10.1
	IV	41	175.0	71.7	1.85	253	1757	24.5	73.2	39.6	+6.4	+3.3
	V	41	175.5	70.8	1.85	238	1654	23.4	68.9	37.2	+0.8	−3.0
	VI	43	178.0	70.1	1.85	233	1622	23.1	67.6	36.5	−0.5	−4.9
	VII	44	165.5	56.5	1.60	204	1421	25.2	59.2	37.0	+3.6	−3.5
	VIII	44	163.0	54.2	1.57	191	1325	24.4	55.2	35.2	−0.4	−8.8
	IX	44	175.0	98.7	2.14	251	1747	17.7	72.8	34.2	−12.7	−12.0
	X	44	179.5	70.8	1.88	219	1522	21.5	63.4	33.7	−7.2	−13.6
			Average		1.819				Average		+1.01	−3.2
45–49	XI	45	175.5	63.7	1.77	217	1508	23.7	62.7	35.4	−0.5	−6.8
	XII	45	180.0	70.6	1.88	243	1688	23.9	70.3	37.4	+3.3	−1.1
	XIII	45	172.0	80.1	1.93	201	1397	17.4	58.2	30.2	−19.0	−25.0
	XIV	46	171.0	60.7	1.69	217	1508	24.8	62.8	37.2	+4.3	−1.6
	XV	46	173.5	75.0	1.88	234	1626	21.7	67.8	36.1	−1.8	−4.7
	XVI	47	180.0	70.2	1.88	219	1522	21.7	63.4	33.7	−5.8	−12.2
	XVII	47	181.0	74.6	1.94	263	1827	24.5	76.1	39.2	+8.8	+3.6
	XVIII	48	165.0	55.4	1.59	214	1487	26.8	62.0	36.0	+11.9	−5.0
	XIX	48	177.5	76.2	1.93	231	1605	21.1	66.9	33.6	−4.3	−12.5
	XX	49	169.0	57.3	1.65	207	1440	25.1	60.0	36.9	+5.2	−2.4
			Average		1.816				Average		+0.21	−6.8
50–54	XXI	50	179.0	78.5	1.96	205	1424	18.1	59.3	30.3	−16.4	−18.5
	XXII	51	177.5	70.7	1.87	246	1709	24.2	71.3	38.1	+8.0	+2.4
	XXIII	51	173.0	56.5	1.65	212	1473	26.1	61.3	37.2	+8.0	0
	XXIV	51	161.0	69.0	1.72	198	1376	19.9	57.4	33.4	−6.8	−10.2
	XXV	51	165.0	65.0	1.71	195	1358	20.9	56.6	33.2	−5.8	−10.8
	XXVI	53	169.5	70.4	1.80	221	1536	21.8	64.1	35.6	+0.7	−4.3
	XXVII	53	175.0	75.8	1.90	226	1570	20.7	65.4	34.4	−3.5	−7.5
	XXVIII	53	169.0	47.5	1.52	192	1334	28.1	55.5	36.5	+10.4	−1.9
	XXIX	53	172.0	81.6	1.94	288	2001	24.5	83.3	42.9	+18.3	+15.3
	XXX	54	171.0	59.0	1.68	226	1570	26.6	65.3	38.9	+14.7	+4.6
			Average		1.775				Average		+2.8	−3.1
55–59	XXXI	55	179.0	77.0	1.94	209	1452	18.9	60.5	31.2	−11.9	−14.8
	XXXII	55	175.5	61.0	1.74	203	1410	23.1	58.8	33.8	−0.1	−7.7
	XXXIII	55	176.5	80.0	1.96	222	1542	19.3	64.3	32.8	−8.1	−10.4
	XXXIV	56	178.0	58.0	1.72	177	1230	21.2	51.3	29.8	−10.6	−18.6
	XXXV	57	163.5	76.5	1.81	222	1542	20.2	64.3	35.5	−0.6	−3.0
	XXXVI	58	177.5	61.8	1.77	201	1397	22.6	58.3	32.9	−1.1	−10.0
	XXXVII	58	172.0	71.6	1.83	194	1348	18.8	56.2	30.2	−11.3	−17.5

TABLE 2—*Continued*

GROUP	SUBJECT NO.	AGE	HEIGHT	BODY WEIGHT (WITHOUT CLOTHING)	BODY SURFACE AREA (DU BOIS WEIGHT-HEIGHT CHART)	OXYGEN CONSUMPTION, 0°C., 760 MM. HG.	METABOLISM				DIFFERENCE FROM CURVES OF	
							Total	Per kgm.	Total	Per sq. meter body surface	Harris Benedict (1919)	Du Bois Boothby Sandiford (1929)
							Calories per 24 hours		Calories per hour			
		yrs.	*cm.*	*kgm.*	*sq. meter*	*cc.*					*per cent*	*per cent*
55–59	XXXVIII	59	178.0	76.6	1.90	258	1793	23.4	74.8	39.4	+11.2	+7.7
Cont'd	XXXIX	59	171.5	72.8	1.84	184	1278	17.6	53.3	29.0	−16.3	−20.8
	XL	59	178.5	87.6	2.06	245	1702	19.4	70.8	34.4	−3.6	−6.0
			Average		1.857				Average		−5 2	−10 1
60–64	XLI	60	169.0	65.7	1.74	220	1529	23.3	63.8	36.7	+8.4	+1.9
	XLII	60	172.5	61.8	1.72	202	1403	22.7	58.6	34.7	+2.2	−3.7
	XLIII	61	166.5	67.4	1.74	211	1466	21.8	61.2	35.7	+3.8	−0.8
	XLIV	61	171.5	71.8	1.83	230	1598	22.3	66.7	36.4	+6.5	+1.1
	XLV	61	173.0	67.0	1.78	215	1494	22.4	62.2	34.9	+3.6	−3.2
	XLVI	61	179.0	91.0	2.09	237	1647	18.1	68.7	32.9	−8.6	−9.4
	XLVII	63	173.0	87.2	2.00	257	1786	20.5	74.4	37.2	+4.7	+3.2
	XLVIII	63	172.0	71.0	1.82	208	1445	20.4	60.2	33.1	−2.2	−8.7
	XLIX	64	172.5	74.8	1.86	226	1570	21.0	65.4	35.2	+3.0	−2.3
	L	64	174.0	54.4	1.65	188	1306	24.0	54.4	32.1	+4.2	−12.1
	LI	64	167.5	51.7	1.57	187	1299	25.1	54.1	34.5	+9.9	−4.3
	LII	64	164.0	76.6	1.82	205	1424	18.6	59.2	32.5	−5.6	−10.8
	LIII	64	183.0	58.2	1.75	193	1341	23.0	55.9	31.9	−0.7	−12.8
			Average		1.798				Average		+2.2	−4.8
65–69	LIV	65	174.0	80.0	1.94	239	1661	20.8	69.2	35.7	+3.9	+1.0
	LV	65	165.0	63.2	1.68	195	1355	21.4	56.4	33.4	+2.5	−5.7
	LVI	66	170.5	67.6	1.77	233	1619	23.9	67.4	38.1	+15.4	+7.4
	LVII	66	168.5	61.6	1.69	218	1515	24.6	63.2	37.4	+15.6	+5.6
	LVIII	67	182.0	87.8	2.09	249	1730	19.7	72.1	34.5	−0.1	−2.3
	LIX	68	170.0	51.0	1.58	214	1487	29.2	61.9	39.1	+28.3	+9.7
	LX	69	167.5	57.3	1.64	205	1424	24.9	59.2	36.1	+16.2	+2.2
			Average		1.770				Average		+11.7	+2 6
70–74	LXI	70	169.5	66.2	1.75	212	1473	22.3	61.3	35.0		(+0.6*
	LXII	70	175.5	80.5	1.96	239	1661	20.6	69.2	35.3		+1.4
	LXIII	70	153.0	59.0	1.55	169	1174	19.9	49.0	31.6		−11.3
	LXIV	71	168.0	84.0	1.93	245	1702	20.3	71.0	36.8		+5.4
	LXV	71	160.0	61.0	1.63	193	1341	22.0	56.0	34.4		−1.1
	LXVI	71	161.0	57.5	1.59	192	1334	23.2	55.5	34.9		+0.3
	LXVII	71	166.0	56.8	1.62	196	1362	24.0	56.8	35.1		+0.9
	LXVIII	72	172.0	81.2	1.94	239	1661	20.4	69.3	35.7		+2.5
	LXIX	72	165.0	48.6	1.52	191	1327	27.3	55.2	36.3		+4.1
	LXX	72	176.0	69.5	1.84	179	1244	17.9	51.7	28.1		−23.8
	LXXI	72	164.5	57.2	1.62	200	1390	24.3	57.9	35.7		+2.5
	LXXII	73	171.0	80.8	1.92	248	1723	21.3	71.7	37.3		+6.7
	LXXIII	74	172.5	68.6	1.804	193	1341	19.5	56.0	31.1		−11.9
			Average		1 744				Average			−1 8

TABLE 2—*Concluded*

GROUP	SUBJECT NO.	AGE†	HEIGHT	BODY WEIGHT (WITHOUT CLOTHING)	BODY SURFACE AREA (DU BOIS WEIGHT-HEIGHT CHART)	OXYGEN CONSUMPTION, (0°C., 760 MM. HG.	METABOLISM				DIFFERENCE FROM CURVES OF	
							Total	Per kgm.	Total	Per sq. meter body surface	Harris Benedict (1919)	Du Bois Boothby Sandiford (1929)
							Calories per 24 hours		Calories per hour			
		yrs.	*cm.*	*kgm.*	*sq. meter*	*cc.*					*per cent*	*per cent*
75–79	LXXIV	75	173.5	77.4	1.91	232	1612	20.8	67.3	35.2		+2.8
	LXXV	75	164.5	67.8	1.72	205	1424	21.0	59.3	34.5		+0.9
	LXXVI	76	174.5	65.5	1.78	202	1403	21.4	58.5	32.9		−4.0
	LXXVII	77	167.0	69.6	1.77	204	1417	20.4	59.2	33.4		−2.4
	LXXVIII	77	161.0	65.0	1.68	199	1383	21.3	57.7	34.3		+0.3
	LXXIX	77	166.0	73.8	1.82	217	1508	20.4	62.9	34.6		+2.9
	LXXX	78	163.0	63.5	1.68	211	1466	23.1	61.1	36.4		+6.0)
			Average		1.766						Average	+0.9
80–84	LXXXI	80	171.0	64.0	1.74	170	1181	18.5	49.2	28.3		
	LXXXII	80	167.0	67.2	1.74	199	1383	20.6	57.6	33.1		
	LXXXIII	80	161.0	43.6	1.42	159	1105	25.3	45.9	32.3		
	LXXXIV	80	176.0	64.0	1.78	210	1459	22.8	60.8	33.6		
	LXXXV	81	165.5	72.6	1.79	237	1647	22.7	68.6	38.3		
	LXXXVI	81	159.5	65.1	1.66	190	1320	20.3	55.0	33.1		
	LXXXVII	82	184.0	76.8	1.98	261	1813	23.6	75.7	38.2		
	LXXXVIII	82	166.0	71.8	1.79	223	1549	21.6	64.5	36.0		
	LXXXIX	82	160.0	52.8	1.53	146	1015	19.2	42.3	27.6		
	XC	82	172.5	51.2	1.60	198	1376	26.9	57.3	35.8		
	XCI	82	153.5	45.0	1.38	158	1098	24.4	45.7	33.1		
	XCII	83	169.0	80.9	1.91	230	1598	19.8	66.6	34.9		
	XCIII	84	180.0	72.6	1.90	226	1570	21.6	65.4	34.4		
	XCIV	84	163.0	57.7	1.61	186	1292	22.4	53.9	33.5		
			Average		1.702							
85–89	XCV	85	168.5	56.2	1.63	177	1230	21.9	51.2	31.4		
	XCVI	85	162.5	53.8	1.55	203	1410	26.2	58.9	38.0		
	XCVII	85	168.0	62.8	1.71	190	1320	21.1	55.0	32.2		
	XCVIII	85	175.5	55.0	1.67	195	1355	24.6	56.4	33.8		
	XCIX	89	160.0	55.6	1.68	194	1348	24.2	56.3	33.5		
	C	89	163.0	58.4	1.61	176	1223	20.9	51.0	31.6		
			Average		1.642							
90–101	CI	91	179.0	72.6	1.90	198	1376	19.0	57.2	30.1		
	CII	91	167.0	61.6	1.68	177	1230	20.0	51.2	30.5		
	CIII	101	166.0	45.8	1.47	146	1014	22.1	42.2	28.7		
			Average		1.683							

† Age as of last birthday.

* The data in this column result from extrapolation of the curve of Du Bois modified by Boothby and Sandiford (1929).

straight line if the measurement is taken of the entire population between ages 40 and 89. The progressive decline is expressed by a regression curve which is derived from the use of the equation:[3]

$$\text{Calories} = M_c + r_{AC}\frac{\sqrt{\Sigma C^2 - (\Sigma C)(M_c)}}{\sqrt{\Sigma A^2 - (\Sigma A)(M_A)}}(A - M_A)$$

in which C = calories per square meter per hour; A = age in years; M = mean. Since r (the coefficient of correlation) is negative the solution of this equation is:

$$\bar{C} = 39.138 - 0.0678 \times A^4$$

When $A = 40$, $\bar{C} = 36.426$; and
$A = 89$, $\bar{C} = 33.106$ (fig. 1).

For the 50 year period the regression is accordingly 3.32 calories or 9.1 per cent. For each of the five decades beginning with age 40 it is 0.664

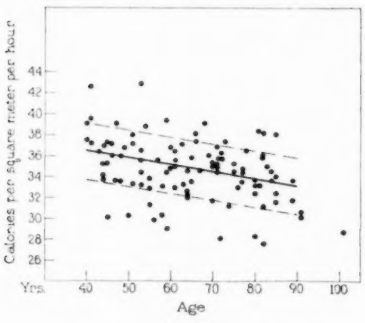

Fig. 1

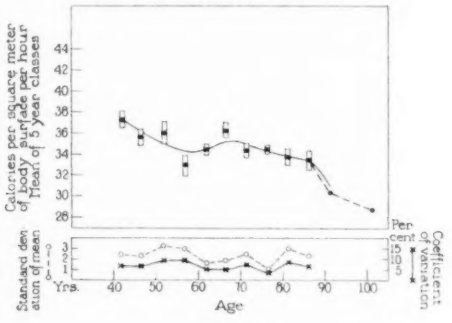

Fig. 2

Fig. 1. Basal metabolism in normal men over 40 years of age.

● individual observation.

——— regression of metabolism on age of 100 men, aged 40 to 89 years, twenty in each decade, with standard error of estimate ----------.

Fig. 2. Basal metabolism in normal men over 40 years of age grouped according to half decades of age.

Hollow rectangles with solid dots represent the mean of 5 year classes, ± standard error of mean of age and of metabolism.

——— curve smoothed according to $\frac{N^a a + 2N^b b + N^c c.}{N^a + 2N^b + N^c}$

(Allowance for number of observations in each group.)

[3] For the methods of statistical analysis employed in this and subsequent papers see Fisher (11), Wallace and Snedecor (12), and Ezekiel (13).

[4] If, instead of a uniform distribution of 20 men per decade, the whole experience ranging from 40 to 101 years is taken, $C = 39.481 - 0.0744 \times A$. In this case the curve is slightly steeper (fig. 4).

calories. Since the coefficient of correlation of metabolism and age is -0.3328 ± 0.0889 a highly significant negative correlation appears to exist (table 3).

c. Though the curve just given represents the course of events as an expression of the trend between ages 40 and 89, the facts actually require

TABLE 3

Summary of results of the statistical analysis of the basal metabolism, based on the data in table 2

AGE			BASAL METABOLISM, CALORIES PER SQUARE METER PER HOUR									
Group	Mean	NUMBER OF SUBJECTS	Minimum	Maximum	Mean M_c	Standard deviation σ_c	Standard error of mean σM_c	COEFFICIENT OF VARIATION $\frac{100\sigma_c}{M_c}$	COEFFICIENT OF CORRELATION r_{AC}	STANDARD ERROR OF CORRELATION (r_{AC}) σ_r	VALUE OF t	LEVEL OF SIGNIFICANCE FISHER'S TABLES
yrs.	*yrs.*							*per cent*				
40–44	42.20	10	33.7	42.6	37.3	2.55	0.848	6.8	−0 7305	±0.1554	4.7	
45–49	46.60	10	30.2	39.2	35.6	2.40	0.800	6.8	+0.1959	±0.3205	0.6	
50–54	52.00	10	30.3	42.9	36.1	3.35	1.115	9.3	+0.5837	±0.2198	2.7	
55–59	57.10	10	29.0	39.4	32.9	2.95	0.984	9.0	+0.2190	±0.3173	0.7	
60–64	62.31	13	31.9	37.2	34.5	1.73	0.503	5.0	−0.4994	±0.2167	2.3	
65–69	66.67	7	33.4	39.1	36.3	1.86	0.761	5.1	−0.3675	±0.3532	1.0	
70–74	71.46	13	28.1	37.3	34.4	2 50	0.723	7.3	−0.1239	±0.2842	0 4	
75–79	76.43	7	32.9	36.4	34.5	1.06	0.433	3.1	+0.2562	±0.3815	0.7	
80–84	81.64	14	27.6	38.3	33.7	2.96	0.820	8.6	+0.2395	±0.2614	0.9	
85–89	86.33	6	31.4	38.0	33.4	2.24	1.002	6.7	−0.2747	±0.4204	0.7	
90–94	91.00	2	30.1	30.5	30.3	0.20	0.200	0.7	0	0	0	
101		1	28.7									
40–49	44.40	20	30.2	42.6	36.4	2.62	0.601	7.2	−0.4439	±0.1842	2.4	
50–59	54.55	20	29.0	42.9	34.5	3.53	0.809	10.2	−0.2181	±0.2185	1.0	
60–69	63.80	20	31.9	39.1	35.1	1.99	0.451	5.7	+0.2501	±0.2151	1.2	
70–79	73.20	20	28.1	37.3	34.4	2.11	0.485	6.1	−0.0110	±0.2294	0.005	
80–89	83.05	20	27.6	38.0	33.6	2.77	0.634	8.2	+0.0018	±0.2294	0.0008	
90–101	94.33	3	28.7	30.5	29.8	0.77	0.546	2.6	−0.7336	±0.3267	2.2	
40–64	52.63	53	29 0	42.6	35.2	2.99	0.410	8.5	−0.3403	±0.1215	2.8	Significant
65–89	76.40	47	27.6	39.1	34.4	2.54	0.371	7.4	−0.3427	±0.1287	2.7	Significant
65–101	77.48	50	27.6	39.1	34.1	2.71	0.383	7.9	−0.4439	±0.1136	3.9	Highly significant
40–89	63.80	100	27 6	42.6	34.8	2.82	0.282	8.1	−0.3328	±0 0889	3.7	Highly significant
40–101	64 69	103	27.6	42.6	34.7	2.91	0.286	8.4	−0.3735	±0.0848	4.4	Highly significant

a more complex presentation if the mean levels at succeeding decades or half-decades are taken into account. It has been necessary to learn, therefore, what the average basal rate is in each successive age group. The mean values have accordingly been ascertained decade by decade and at half-decade intervals (table 3).

The decline is less orderly if the averages obtained in five-year groups are plotted (fig. 2), more orderly if those of ten-year periods (fig. 3) are used. At 40 to 44 years, the mean value was 37.28 calories per square meter per hour, and at 45 to 49, 50 to 54, 65 to 69 years it was approximately 36 calories, but at 55 to 59 years an unexpected irregularity brought about a fall in the curve to 32.9 calories. After age 70 the mean values were found progressively to fall.

If, instead of the means at five-year intervals, those of whole decades are plotted, the resulting curve takes on a more orderly or consistent ap-

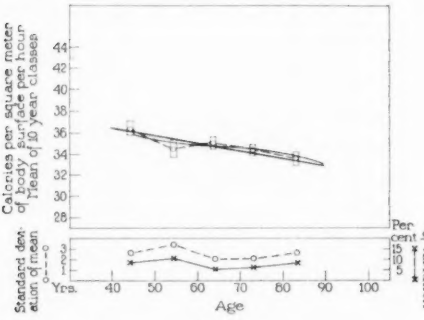

Fig. 3

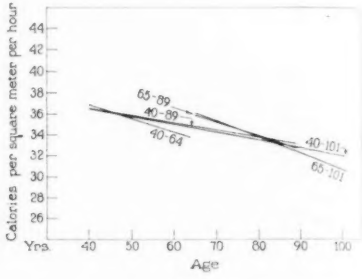

Fig. 4

Fig. 3. Basal metabolism in normal men over 40 years of age grouped according to decades of age.

Hollow rectangles with open dots represent the mean of 10 year classes, twenty men in each class, ± standard error of mean.

——— curve of 10 year means smoothed according to $\frac{a + 2b + c}{4}$.

——— curve of regression, 40 to 89 years.

Fig. 4. Basal metabolism in normal men over 40 years of age. Curves of regression of various age groups.

pearance (fig. 3). There is then a decline which is gradual through the 6th, 7th and 8th decades.

It is improbable that the curve as now presented is definitive.[5] It seems necessary to believe a smooth curve is descriptive of the course of events.

From what is already known of changes in the rate of metabolism it is not unexpected that after age 40 the slope of the curve which describes them undergoes inflection. It is only in the earliest years that the level rises sharply. Afterward it exhibits declines, at various rates, or steadiness. The declines are marked in childhood (ages 2 to 12) and again after

[5] This situation is not unique. See Harris and Benedict (7), pp. 120-122, and Du Bois (4) pp. 126-128.

adolescence (4, 10, 14, 15, 16). During adolescence the rate of decline may be the same (14, 15, 16), or more probably arrested (1, 4, 17, 18, 19). The period of greatest steadiness is found between 20 years and 40 years though here also there is a slight decline. A decline then sets in again about the exact nature of which there is doubt. As the result of this study no more can be definitely inferred than that the slope of the curve of regression undergoes one or more inflections between its end points

TABLE 4

*Basal metabolism in normal adult men by half-decades of age between 40 and 104 years**

1	2	3	4	5	6
AGE GROUP	BASAL METABOLISM, CALORIES PER SQUARE METER PER HOUR		CHANCES OF BEING NORMAL WHEN VARIATION FROM MEAN IS MORE THAN		
	Mean	Standard error of estimate S	1S = 1 in 3	2S = 1 in 20	2½S = 1 in 100
yrs.			*per cent*	*per cent*	*per cent*
40–44	36.4	2.69	7.5	15.0	18.5
45–49	36.1	2.69	7.5	15.0	19.0
50–54	35.7	2.69	7.5	15.0	19.0
55–59	35.4	2.69	7.5	15.0	19.0
60–64	35.0	2.69	7.5	15.5	19.5
65–69	34.7	2.69	8.0	15.5	19.5
70–74	34.4	2.69	8.0	15.5	19.5
75–79	34.0	2.69	8.0	16.0	20.0
80–84	33.7	2.69	8.0	16.0	20.0
85–89	33.3	2.69	8.0	16.0	20.0
90–94	32.6	2.73	8.5	17.0	21.0
95–99	32.3	2.73	8.5	17.0	21.0
100–104	31.9	2.73	8.5	17.0	21.0

* The results from 40 to 89 are calculated with the use of the equation:

$$\text{Calories per square meter per hour} = 39.138 - 0.0678 \times \text{Age};$$

and from 90 to 104 years with the use of the equation:

$$\text{Calories} = 39.481 - 0.0744 \times \text{Age}$$

The chances of being normal are given when the variation from the average value (column 2) is greater than one standard error of estimate (1 chance in 3, column 4); 2 times the standard error of estimate (1 chance in 20, column 5); and $2\frac{1}{2}$ times the standard error of estimate (1 chance in 100, column 6).

(40 and 89 years) (fig. 4). The accumulation of more measurements should make it possible to define with reasonable accuracy precisely what the course of the curve actually is in the long period of decline from 40 to 90. There are of course a number of possibilities: *1*, that the plateau of middle life is prolonged; *2*, that it descends in a fashion illustrated by an S-shaped curve; *3*, that after a period of 15 years, until 55 years, it remains level, thence to descend in a manner illustrated by a parabolic curve.

The values given in the equation in paragraph *b* are recapitulated in

table 4. The standard error of estimate for the men, 40 to 89 years of age, is 2.69 calories per square meter per hour. The chances of being average are given in table 4 and figure 6.

Discussion. The state of knowledge of the changes in the rate of metabolism with age, at least in the later decades, is not yet in a satisfactory situation as a study of all the measurements over 40 years of age now available indicates (table 1).

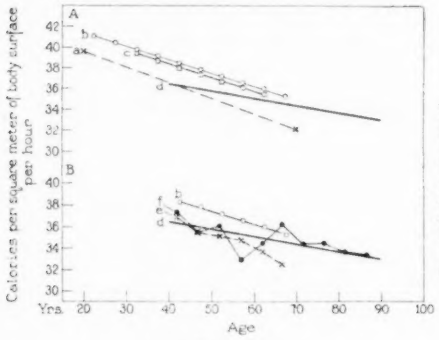

Fig. 5

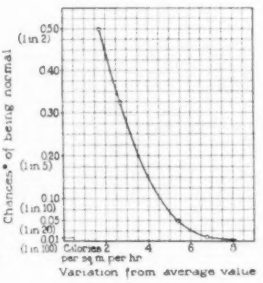

Fig. 6

Fig. 5. *A*. Comparison of the regression curves obtained from the present study with those of Harris and Benedict (1919) and of Du Bois as modified by Boothby and Sandiford (1929), and Boothby, Berkson, and Dunn (1936).

a, regression by prediction equation, Harris and Benedict (1919).

b, regression by 5 year means, Boothby and Sandiford (1929).

c, regression by 5 year means, Boothby, Berkson, and Dunn (1936).

d, regression by prediction equation, this study.

B. Curves based on the present study of 5 year groups.

b, as in *A*.

d, as in *A*.

e, regression by 5 year means for subjects of this study calculated from tables by Harris and Benedict (1919).

f, regression by 5 year means, this study.

Fig. 6. A curve showing the probability that the observed basal metabolism is normal expressed as variations from the average value in men over 40 years of age.

* The phrase used for this expression by Ezekiel (1930) is the "probability of specified departure" from the average value.

Note: When in an individual the basal rate differs 6.7 calories from the average the chances are only 1:100 that this measurement is normal.

When the straight curve resulting from this analysis is compared with that of Du Bois as modified by Boothby and Sandiford (16), and by Boothby, Berkson, and Dunn (20) and with that of Harris and Benedict (7), its slope is less steep than that of the other two,—0.7 calorie per square meter per hour against theirs of 1.3, and lies about mid-way between

the two (fig. 5).[6] The divergence of the Harris-Benedict curve is marked in the later years. The cause of this is not quite certain, but it may depend on the fact that their sample contained predominantly young subjects. There were only eleven individuals over age 40 and none older than 64. There exists also the factor of greater variation in the metabolism of elderly subjects, noted again by Du Bois (4) and Benedict (21) after further recent experience.

Variations such as these recall the statement of Gephart and Du Bois (22)[7] that "the selection of a proper normal base-line is a matter of great difficulty" and the one of Harris and Benedict (7)[8] that "the difficulties associated with variations in basal metabolism suggest that the difficulties of the selection of proper controls have been underestimated rather than overestimated in the past."

CONCLUSIONS

1. The basal metabolic rates have been studied in 100 normal men, 20 in each decade from 40 to 89 years. In addition measurements were obtained of 2 men at 91 years, and one at 101 years. It was found that the rate of metabolism falls between 40 and 89 and that the fall can be expressed by the equation: The calories lost per square meter per hour $= 39.138 - 0.0678 \times$ age. The negative correlation is $- 0.3328 \pm 0.0889$ and is highly significant. In each decade, the regression is 0.664 calorie per square meter per hour.

2. The mean values in succeeding decades suggest that the rate of decline of the curve is not straight but may be arrested between 50 years and 79 years. The curve obtained is not smooth; were more than 20 individuals examined in each decade it might become so. That variation in each age group exists has been learned again in this investigation. In any 5 or 10 year group the maximum deviations from the respective means were -18.3 and $+24.4$ per cent. The standard deviation, the standard error of the mean and the coefficient of correlation have therefore been calculated in 5, 10 and 25 year groups.

For the entire series (40 to 101) the mean is 34.667 calories per square meter per hour; the standard deviation is 2.906 (8.4 per cent of the mean) and the standard error 0.286 calories. For the evenly distributed group (40 to 89) the mean is 34.814, the standard deviation 2.813 (8.1 per cent

[6] Du Bois (4), 3rd ed. revised, 1936, p. 171. "We may conclude that there is a great deal of variation in old people, probably depending upon the degree of senility, and that if the general slope of the lines of the standards are continued into old age, the Harris-Benedict level is somewhat too low and the Aub and Du Bois and Boothby a little too high."

[7] P. 857.

[8] P. 224.

of the mean), the standard error of the mean 0.282, and the standard error of estimate 2.69 calories per square meter per hour.

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CHANGES WITH AGE IN THE CARDIAC OUTPUT IN ADULT MEN[1]

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The maintenance of a usual volume of output[2] of blood per minute from the heart is among the important functions of the circulation. This volume in normal young men and women under so-called basal conditions has been found, in recent years, to be 2.2 ±0.3 liters per square meter of body surface (1). In the same individual under the same conditions the amount is the same from day to day. It is approximately the same, furthermore, in individuals of the same sex and size of body. But whether it remains the same, irrespective of age, is still unknown. A few measurements have been made in children (1b), in whom the index, in keeping with their higher metabolic rate, is relatively higher than in adults. From other publications may be collected the results of a few measurements, made for other purposes, in individuals over forty years of age. But knowledge of the variation of the cardiac index with age is, as Grollman (1b) has pointed out, deficient.

This communication reports the changes in the volume of the index which takes place under basal conditions, in normal men over forty years of age. The subjects were one hundred men from forty to eighty-nine years, twenty in each of the five decades; there were in addition two men of ninety-one, and one of one hundred and one years. They were the same men who were described in the preceding report (2). They were studied under uniform and basal conditions in the manner there described.

METHOD. For measuring the cardiac output the acetylene rebreathing procedure was employed. Accuracy in technique in the use of the method requires: *1*, that complete diffusion of the gas mixture in the lung-bag system be attained before the first sample of gas is taken for analysis; and *2*, that later samples be taken before blood containing acetylene has returned to the lungs. In normal young adults, six deep respirations in 15 seconds have been found sufficient to attain diffusion, and a lapse of

[1] This paper is no. 18 of the series on Physiological Ontogeny.

[2] The phrase "cardiac output" when used in these papers means the amount of blood expelled by the heart in a minute. If the calculation is made in terms of the surface area of the body, the phrase "cardiac index" (*CI*) is used.

eight or ten seconds thereafter sufficient, before return of the blood to the lungs (3, 4). To be certain these conditions are fulfilled, three samples are taken in succession; if the results derived from the first and second samples and from the second and third samples are reasonably similar, the test is regarded as having been correctly made (5) (fig. 1). In this study three or four samples were always taken; those tests were considered satisfactory in which the difference in the result from samples A and B, and that from B and C was less than 12 per cent of the average of A and C. Closer agreement was not found possible. Such checks are especially important in elderly persons. In the first 45 normal subjects the mean

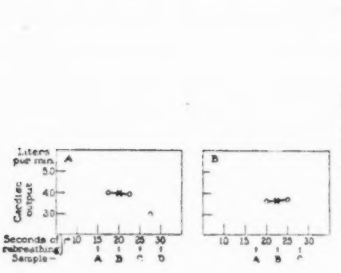

Fig. 1

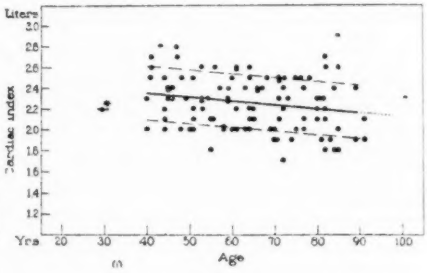

Fig. 2

Fig. 1. The method of control of tests of gas samples for measuring cardiac output.

A. Subject II, 40 years; 4 samples were taken at 15(A), 20(B), 25(C), 30(D) seconds of rebreathing.

B. Subject XCII, 83 years; 3 samples were taken at 17(A), 22(B), 27(C) seconds of rebreathing.

Fig. 2. The cardiac index in normal men over 40 years of age.

● individual observations.

——— regression of cardiac index on age, for 100 men, aged 40 to 89 years, twenty in each decade, extended ---------- to include 3 men over 89 years, with curves - - - - - of standard error of estimate.

*Average in normal men aged 21 to 35 years, Grollman (1).

deviation of the results of samples A and B or B and C from the average of A and C, was 4.7 per cent.

In the early experiments four samples were taken in succession. In a few subjects, measurements were made on succeeding days; no significant deviations appeared.

RESULTS are reported in several ways:[3] *a*. The mean value of the cardiac index in one hundred men from forty to eighty-nine years, separated in decades or half decades, ranged from 2.40 to 2.17 liters; in the series of one hundred men taken as a whole, it was 2.26 liters (table 1, fig. 2). These values resemble the average 2.2 liters found in young men of twenty to

[3] References to the statistical methods employed are given in preceding paper (2).

TABLE 1

Measurements of certain circulatory phenomena: data for the calculation of the cardiac index

GROUP	SUBJECT NO.	PULSE RATE PER MINUTE	OXYGEN CONSUMPTION PER MINUTE (WITHOUT CORRECTION FOR BAROMETER AND TEMPERATURE)	ARTERIO-VENOUS OXYGEN DIFFERENCE PER LITER OF BLOOD	CARDIAC OUTPUT PER MINUTE	CARDIAC INDEX (OUTPUT PER SQUARE METER OF BODY SURFACE PER MINUTE)	SYSTOLIC OUTPUT	SYSTOLIC OUTPUT PER SQUARE METER OF BODY SURFACE	BLOOD PRESSURE, MILLIMETERS Hg				X-RAY OF HEART	
									Systolic		Diastolic			
									Right arm	Left arm	Right arm	Left arm	Transverse diameter	Cardiac area
			cc.	*cc.*	*liters*	*liters*	*cc.*	*cc.*					*cm.*	*sq. cm.*
40–44	I	56	283	72.6	3.9	2.0	70	35	118	110	78	72	14.80	107.2
	II	70	253	64.2	3.9	2.3	56	33	110	100	76	70	13.91	129.2
	III	56	286	68.6	4.8	2.7	86	48	104	96	62	54	14.31	115.5
	IV	62	284	57.6	4.9	2.6	79	42	128	118	80	76	13.30	122.5
	V	66	265	58.2	4.6	2.5	71	38	136	120	0	80	12.10	84.9
	VI	60	259	50.6	5.1	2.8	85	45	115	115	70	64	13.50	128.7
	VII	58	233	58.3	4.0	2.5	69	43	100	90	70	70	12.80	98.7
	VIII	64	211	61.7	3.4	2.2	53	33	112	114	70	70	12.39	107.5
	IX	56	274	65.8	4.2	2.0	75	35	126	110	92	80	15.80	109.2
	X	66	242	62.7	3.9	2.1	59	31	110	100	76	70	14.0	111.9
45–49	XI	58	235	58.4	4.0	2.3	69	39	124	140	84	86	12.38	100.1
	XII	68	265	57.7	4.6	2.4	68	36	130	120	82	86	11.90	94.1
	XIII	54	221	49.4	4.5	2.3	83	43	136	106	82	80	15.30	107.5
	XIV	66	243	57.3	4.2	2.5	64	38	122	114	80	70	14.30	104.2
	XV	56	263	60.3	4.4	2.3	79	42	106	106	76	76	15.10	123.1
	XVI	70	245	47.7	5.1	2.7	73	39	110	90	62	56	14.10	108.7
	XVII	60	295	54.6	5.4	2.8	90	46	116	100	70	68	15.40	117.9
	XVIII	54	214	65.9	3.2	2.0	59	37	124	116	86	82	11.99	108.9
	XIX	62	257	55.2	4.7	2.4	76	39	120	106	80	72	14.95	88.7
	XX	60	225	60.5	3.7	2.3	62	37	118	120	80	78	11.80	88.4
50–54	XXI	62	231	58.6	3.9	2.0	63	32	104	108	62	72	13.80	99.6
	XXII	64	270	69.4	3.9	2.1	61	33	154	150	88	90	12.95	91.9
	XXIII	55	238	56.9	4.2	2.5	76	46	110	110	60	60	11.41	113.5
	XXIV	66	218	58.0	3.8	2.2	58	34	148	148	90	90	14.80	122.9
	XXV	58	218	63.6	3.4	2.0	59	34	120	115	80	60	13.80	106.9
	XXVI	82	248	58.6	4.1	2.2	50	28	116	116	74	76	14.05	96.9
	XXVII	57	257	59.8	4.3	2.3	75	39	130	120	85	80	14.50	95.7
	XXVIII	60	214	55.3	3.9	2.6	65	43	110	110	85	80	10.99	99.2
	XXIX	54	311	71.2	4.4	2.3	81	42	125	112	70	70	15.00	123.2
	XXX	60	253	65.5	3.9	2.3	65	39	110	100	74	64	11.65	115.1

TABLE 1—*Continued*

GROUP	SUBJECT NO.	PULSE RATE PER MINUTE	OXYGEN CONSUMPTION PER MINUTE (WITHOUT CORRECTION FOR BAROMETER AND TEMPERATURE)	ARTERIO-VENOUS OXYGEN DIFFERENCE PER LITER OF BLOOD	CARDIAC OUTPUT PER MINUTE	CARDIAC INDEX (OUTPUT PER SQUARE METER OF BODY SURFACE PER MINUTE)	SYSTOLIC OUTPUT	SYSTOLIC OUTPUT PER SQUARE METER OF BODY SURFACE	BLOOD PRESSURE, MILLIMETERS Hg				X-RAY OF HEART	
									Systolic		Diastolic			
									Right arm	Left arm	Right arm	Left arm	Transverse diameter	Cardiac area
			cc.	*cc.*	*liters*	*liters*	*cc.*	*cc.*					*cm.*	*sq. cm.*
55–59	XXXI	60	232	65.4	3.5	1.8	58	30	160	130	108	90	15.00	118.2
	XXXII	57	224	62.4	3.6	2.1	63	36	120	116	80	76	12.81	107.8
	XXXIII	63	252	59.5	4.2	2.1	67	34	108	102	66	66	14.50	102.5
	XXXIV	72	200	45.2	4.4	2.6	61	35	104	100	70	72	12.60	113.9
	XXXV	48	251	56.4	4.4	2.4	92	51	90	100	68	68	14.10	101.3
	XXXVI	64	224	62.2	3.6	2.0	56	32	140	118	78	70	12.39	105.2
	XXXVII	56	216	58.9	3.7	2.0	66	36	124	110	96	74	14.00	89.9
	XXXVIII	48	295	66.8	4.4	2.3	92	48	126	116	86	86	15.60	105.2
	XXXIX	57	204	47.7	4.3	2.3	75	41	130	128	82	80	13.70	96.9
	XL	74	275	56.2	4.9	2.4	66	32	156	152	94	90	15.60	83.7
60–64	XLI	50	245	55.6	4.4	2.5	88	51	106	106	70	70	13.40	106.5
	XLII	62	224	63.2	3.5	2.0	56	33	110	100	70	64	12.51	109.2
	XLIII	72	232	50.7	4.6	2.6	64	37	140	128	90	78	13.70	93.5
	XLIV	78	255	54.3	4.7	2.6	60	33	170	150	90	86	14.40	95.2
	XLV	58	237	61.3	3.9	2.2	67	38	140	130	80	86	14.75	129.2
	XLVI	64	263	62.3	4.2	2.0	66	32	114	102	76	66	16.25	125.5
	XLVII	68	290	60.2	4.8	2.4	71	36	120	104	70	60	15.01	96.2
	XLVIII	52	229	66.0	3.5	2.0	67	37	134	126	82	84	14.80	111.9
	XLIX	54	244	60.3	4.0	2.2	74	40	116	110	80	70	15.20	102.5
	L	60	209	68.2	3.4	2.1	57	35	100	94	66	62	12.05	100.3
	LI	54	209	51.7	4.1	2.6	76	48	102	95	58	50	12.05	100.9
	LII	58	226	61.7	3.7	2.0	64	35	132	116	76	70	15.30	108.1
	LIII	68	212	51.5	4.3	2.5	63	36	128	120	70	78	12.50	118.1
65–69	LIV	52	272	65.2	4.2	2.2	81	42	140	136	80	80	13.78	106.5
	LV	64	213	61.2	3.5	2.1	55	33	146	118	76	60	14.65	116.2
	LVI	52	255	62.6	4.1	2.4	79	45	140	118	88	78	13.90	120.7
	LVII	63	240	59.3	4.1	2.4	65	38	110	120	70	80	12.15	85.4
	LVIII	60	274	54.7	5.0	2.4	83	40	140	140	80	80	15.10	118.8
	LIX	63	238	59.3	4.0	2.5	63	40	140	130	80	72	11.00	98.9
	LX	50	220	68.5	3.2	2.0	64	39	124	126	54	54	14.95	118.6

TABLE 1—*Continued*

GROUP	SUBJECT NO.	PULSE RATE PER MINUTE	OXYGEN CONSUMPTION PER MINUTE (WITHOUT CORRECTION FOR BAROMETER AND TEMPERATURE)	ARTERIO-VENOUS OXYGEN DIFFERENCE PER LITER OF BLOOD	CARDIAC OUTPUT PER MINUTE	CARDIAC INDEX (OUTPUT PER SQUARE METER OF BODY SURFACE PER MINUTE)	SYSTOLIC OUTPUT	SYSTOLIC OUTPUT PER SQUARE METER OF BODY SURFACE	BLOOD PRESSURE, MILLIMETERS Hg				X-RAY OF HEART	
									Systolic		Diastolic		Transverse diameter	Cardiac area
									Right arm	Left arm	Right arm	Left arm		
			cc.	*cc.*	*liters*	*liters*	*cc.*	*cc.*					*cm.*	*sq. cm.*
70–74	LXI	52	234	69.6	3.4	1.9	65	37	120	118	68	64	14.10	87.5
	LXII	58	275	72.5	3.8	1.9	66	34	128	136	80	80	15.90	112.4
	LXIII	66	183	60.4	3.0	2.0	45	29	150	136	78	60	11.90	65.1
	LXIV	56	271	66.1	4.1	2.1	73	38	176	130	80	60	14.95	99.8
	LXV	66	218	54.8	4.0	2.5	61	37	160	150	80	88	12.50	81.5
	LXVI	60	209	56.4	3.7	2.3	62	39	114	110	74	70	14.00	98.3
	LXVII	64	218	53.6	4.1	2.5	64	39	138	130	76	76	12.85	104.3
	LXVIII	62	267	63.4	4.2	2.2	68	35	130	144	70	70	14.39	95.2
	LXIX	47	213	57.0	3.7	2.4	79	52	136	140	68	70	12.60	104.9
	LXX	56	193	61.9	3.1	1.7	55	30	110	105	60	55	14.45	100.6
	LXXI	56	225	54.4	4.1	2.5	73	45	126	142	64	72	12.80	83.2
	LXXII	66	273	60.8	4.5	2.3	68	35	180	150	100	86	15.65	122.9
	LXXIII	54	211	62.6	3.4	1.9	63	35	170	155	90	90	13.91	120.4
75–79	LXXIV	56	255	53.9	4.7	2.5	84	44	176	160	96	82	14.30	98.5
	LXXV	54	230	68.1	3.4	2.0	63	37	150	140	90	75	15.20	115.4
	LXXVI	54	227	51.9	4.4	2.5	81	46	140	130	64	64	12.60	82.9
	LXXVII	56	224	60.7	3.7	2.1	66	37	120	130	80	72	13.60	107.2
	LXXVIII	66	221	64.5	3.4	2.0	52	31	150	150	90	80	14.79	100.5
	LXXIX	50	243	54.6	4.4	2.4	88	48	168	126	80	64	14.20	106.4
	LXXX	76	234	55.3	4.2	2.5	55	33	170	170	98	100	13.40	101.5
80–84	LXXXI	50	190	48.9	3.9	2.3	78	45	150	140	80	80	15.20	115.4
	LXXXII	60	218	56.3	3.9	2.2	65	37	180	160	80	82	14.99	103.2
	LXXXIII	62	176	52.8	3.3	2.3	53	37	150	148	74	78	12.20	113.9
	LXXXIV	57	214	63.1	3.7	2.1	65	37	110	112	50	46	12.40	99.1
	LXXXV	50	259	61.1	4.2	2.3	84	47	180	180	70	70	17.90	156.6
	LXXXVI	72	207	66.4	3.1	1.9	43	26	150	150	76	76	13.70	90.5
	LXXXVII	72	296	56.7	5.2	2.6	72	36	180	180	90	90	16.40	145.6
	LXXXVIII	74	249	65.4	3.8	2.1	51	28	128	116	70	70	15.70	97.1
	LXXXIX	54	162	58.1	2.8	1.8	52	34	130	120	60	60	13.70	118.7
	XC	62	221	50.8	4.3	2.7	69	43	178	184	84	88	14.10	101.4
	XCI	54	177	56.4	3.1	2.2	57	41	150	136	80	70	14.10	110.5
	XCII	74	259	70.5	3.7	1.9	50	26	170	144	92	80	20.00	157.2
	XCIII	78	254	55.1	4.6	2.4	59	31	140	130	80	80	13.80	110.9
	XCIV	60	203	70.8	2.9	1.8	48	30	160	150	80	76	14.90	97.5

TABLE 1—*Concluded*

GROUP	SUBJECT NO.	PULSE RATE PER MINUTE	OXYGEN CONSUMPTION PER MINUTE (WITHOUT CORRECTION FOR BAROMETER AND TEMPERATURE)	ARTERIO-VENOUS OXYGEN DIFFERENCE PER LITER OF BLOOD	CARDIAC OUTPUT PER MINUTE	CARDIAC INDEX (OUTPUT PER SQUARE METER OF BODY SURFACE PER MINUTE)	SYSTOLIC OUTPUT	SYSTOLIC OUTPUT PER SQUARE METER OF BODY SURFACE	BLOOD PRESSURE, MILLIMETERS Hg				X-RAY OF HEART	
									Systolic		Diastolic		Transverse diameter	Cardiac area
									Right arm	Left arm	Right arm	Left arm		
			cc.	*cc.*	*liters*	*liters*	*cc.*	*cc.*					*cm.*	*sq. cm.*
85–89	XCV	62	202	63.0	3.2	2.0	52	38	130	132	70	72	12.80	109.6
	XCVI	76	228	50.8	4.5	2.9	59	38	140	140	78	78	11.79	82.2
	XCVII	60	209	70.8	3.0	1.8	50	30	195	195	85	85	14.00	100.9
	XCVIII	68	214	49.0	4.4	2.6	65	39	130	124	70	74	11.60	108.7
	XCIX	65	222	53.9	4.1	2.4	63	38	188	190	76	80	12.75	76.3
	C	48	200	64.2	3.1	1.9	65	40	164	140	86	76	13.30	99.9
90–101	CI	60	225	59.9	3.8	1.9	63	33	124	110	70	68	14.90	104.4
	CII	58	200	56.4	3.6	2.1	62	37	170	174	80	82	13.80	101.8
	CIII	56	164	48.8	3.4	2.3	60	41	136	130	64	58	11.70	69.6

thirty years (1). The mean index is slightly higher in this group in the years from 40 to 69 years than was observed by Grollman in young men. After 70 years it is the same.

The range of variation in each age group was approximately the same, except for the wider range in the ninth decade (figs. 3, 4, table 2). The smallest index observed in the entire series was 1.7; the greatest, 2.9 liters. In the entire scatter, the range is greater than ± 0.3 liter, given by Grollman; but Grollman's results were derived from a group of medical students unusually homogeneous as to age and social station. The better trained and the more uniform the group, the more likely is it, natually, to yield uniform values. The range of the scatter in this series may have been due to the random selection of the sample; in any case it was not great. In the three men, ninety-one and one hundred and one years of age, the cardiac indices were 1.9, 2.1 and 2.3 liters.

The standard deviations in the different age groups on the whole varied only moderately (table 2, figs. 3, 4). The largest in the 5-year classes was ± 0.38 liter or ± 16.6 per cent and occurred in the group aged eighty-five to eighty-nine years; otherwise, it varied from ± 7.6 to ± 12.2 per cent. For the one hundred men it was ± 0.26 liter or ± 11.5 per cent of the mean.

b. A curve drawn to represent the facts shows that there is a general trend according to which the cardiac index declines slightly with age when the mean values of the age groups are joined (figs. 3, 4). The decline

TABLE 2

Summary of results of the statistical analysis of the cardiac index, based on the data in table 1

AGE GROUP	NUMBER OF SUBJECTS	CARDIAC INDEX					COEFFICIENT OF VARIATION $\frac{1000\sigma_{CO}}{M_{CO}}$	COEFFICIENT OF CORRELATION $r_{A\cdot CO}$	STANDARD ERROR OF CORRELATION $(r_{A\cdot CO})$ σ_r	VALUE OF t	LEVEL OF SIGNIFICANCE (FISHER'S TABLES)
		Minimum	Maximum	Mean M_{CO}	Standard deviation σ_{CO}	Standard error of mean $\sigma_{M_{CO}}$					
years		*liters*	*liters*	*liters*	*liter*	*liter*	*per cent*				
40–44	10	2.0	2.8	2.37	0.276	0.092	11.6				
45–49	10	2.0	2.8	2.40	0.214	0.071	8.9				
50–54	10	2.0	2.6	2.25	0.186	0.062	8.3				
55–59	10	1.8	2.6	2.20	0.228	0.076	10.4				
60–64	13	2.0	2.6	2.28	0.244	0.071	10.7				
65–69	7	2.0	2.5	2.29	0.173	0.071	7.6				
70–74	13	1.7	2.5	2.17	0.261	0.075	12.1				
75–79	7	2.0	2.5	2.29	0.223	0.091	9.8				
80–84	14	1.8	2.7	2.19	0.267	0.074	12.2				
85–89	6	1.8	2.9	2.27	0.377	0.169	16.6				
90–94	2	1.9	2.1	2.0	0.10	0.10	5.0				
101	1	2.3									
40–49	20	2.0	2.8	2.38	0.248	0.057	10.4				
50–59	20	1.8	2.6	2.22	0.197	0.045	8.9				
60–69	20	2.0	2.6	2.28	0.222	0.051	9.7				
70–79	20	1.7	2.5	2.21	0.255	0.058	11.5				
80–89	20	1.8	2.9	2.21	0.314	0.072	14.2				
90–101	3	1.9	2.3	2.10	0.163	0.116	7.8				
40–89	100	1.7	2.9	2.26	0.261	0.026	11.5	−0.1862	±0.0965	1.9	Not significant
40–101	103	1.7	2.9	2.26	0.261	0.026	11.6	−0.2039	±0.0944	2.2	Significant

appears progressive when the whole population is considered and is then presented by a straight line (fig. 2). If all the cases in the age span forty to eighty-nine years are treated together, the equation for the decline is:

$$\overline{CI} = 2.49 - 0.0035 \times A$$

A = age in years

When $A = 40$, then $\overline{CI} = 2.35$ liters;

$A = 90$, then $\overline{CI} = 2.17$ liters.

The decline during the 50 year period is, accordingly, 0.18 liter or 7.3 per cent. For each decade the decline is accordingly 0.035 liter or 1.5 per cent.

The coefficient of correlation of age and index, -0.1862 ± 0.0965,

indicates that the two follow an inverse relation; but it is not sufficiently high to be significant. If the results of the entire series, including the three men over eighty-nine years, are correlated with age, the coefficient, -0.2039 ± 0.0944, is significant. The regression equation then would be

$$\overline{CI} = 2.49 - 0.0036 \times A$$

When
$$A = 40, \overline{CI} = 2.35 \text{ liters};$$
$$A = 90, \overline{CI} = 2.17 \text{ liters};$$
$$A = 100, \overline{CI} = 2.13 \text{ liters}.$$

The amount and rate of decline is the same as in the curve of 100 men, aged forty to eighty-nine years.

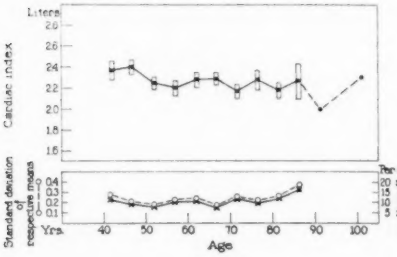

Fig. 3

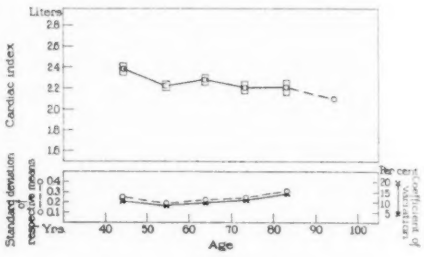

Fig. 4

Fig. 3. Cardiac index in normal men over 40 years of age, divided in 5 year groups.

Hollow rectangles with solid dots represent the mean of 5 year classes, ± standard error of mean of age and of cardiac index.

Fig. 4. Cardiac index in normal men over 40 years of age, divided in 10 year groups.

Hollow rectangles with open dots represent the mean of 10 year classes, ± standard error of mean.

c. Though the general trend of the cardiac index is downward, the mean values if plotted at five or ten year periods indicate that inflections in the curve may exist (figs. 3, 4). The decline appears to be steeper in the forty to fifty-five year period. Then the rate of decline becomes arrested, the decline appearing less orderly when viewed in five year periods, more orderly in ten. The average index in the 20 subjects in the eighth decade and in the 3 subjects over 90 is the same, 2.21 liters. The changes from one to the next period are slight; accordingly the coefficients of correlation are not significant (table 2). The absence of a uniform slope throughout the period forty to one hundred and one suggests, though, that the measurement of more cases is desirable. Since the measurement of the cardiac index in Grollman's method depends on knowing the volume of oxygen consumed, there should be a relation between the decline in the rate of metabolism and a decline in the cardiac index.

The measurement of the volume of the cardiac index, according to the Fick principle, involves the relation of two potentially variable functions, the oxygen consumption or numerator, and the arterio-venous oxygen difference, or denominator, in an equation. The method of calculation reflects the conception that variation in the index follows directly, changes in oxygen consumption; but inversely, changes in the arterio-venous oxygen difference.[4] Since in young men the arterio-venous oxygen difference varies little, the index in them varies directly and quantitatively with oxygen consumption (7, 1a). Should the arterio-venous oxygen difference undergo change in men of older ages, however, the index would no longer be expected to run parallel with oxygen consumption. In the 100 men representing the 50 year period from 40 to 89 years, who form the basis of this study, the arterio-venous oxygen difference declined 3.2

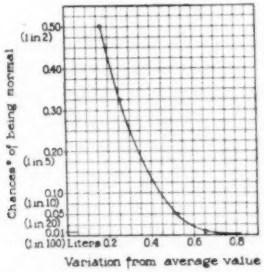

Fig. 5. A curve showing the probability that the observed cardiac index is normal expressed as variations from the average value in men over 40 years of age.

* The phrase used for this expression by Ezekiel (8) is the "probability of specified departure" from the average value.

Note: When in an individual the cardiac index differs 0.65 liter from the average the chances are only 1:100 that this measurement is normal.

per cent, while the consumption of oxygen fell 9.3 per cent. According to the straight line equation for the cardiac index, the decline was 7.3 per cent. The regression of index on age seems to be the result chiefly of the regression in consumption of oxygen.

The standard error of estimate for the men, 40 to 89 years, is 0.259 liter per square meter per minute. The chances of being average depend upon variation from average values based on the standard error of estimate (fig. 5).

Discussion. In view of the very small decline in the cardiac index from forty to eighty-nine years of age, it is clear that the heart can maintain

[4] If like changes occur in consumption and in utilization the cardiac index remains uninfluenced. Unequal changes in the two obviously affect the volume of the cardiac index.

under basal conditions, so far as this function is concerned, a fairly constant level of activity until very late in life. In one man at all events, of 101 years, it met a demand for the same amount of blood as is customary in younger men. Obviously greater experience of this function in men of this age group is necessary than has been gained so far.

It appears that the decline in index occurs not solely as a consequence of age but as a result of the decline with age of the consumption of oxygen. In calculating the "cardiac output" Grollman (1a) has assumed that the surface area provides a suitable object of reference. In any event experience has shown that the index is proportional to the surface area in the same sense as is the consumption of oxygen. These calculations have all assumed constancy in the area, structure, and function of the skin. With this reservation the results are taken to present values for the cardiac index in normal men according to their age. They indicate that the index declines 1.5 per cent per decade.

The changes which have been studied relate solely to the basal state. Naturally, it is not under such conditions that capability is measured. Capability is measured when the body operates under stress. What Meltzer called factors of safety are then brought into play. The influence of exercise, of abnormal affections and of disease requires to be studied before insight into the reserve capacity of the heart is secured. Obviously old men do not and perhaps cannot do what younger ones can—alterations in other circulatory functions may in a collateral way account for changes in the activity of the heart, even under basal conditions.

SUMMARY

1. The cardiac index has been measured in 100 normal men, 20 in each decade from 40 to 89 years, and in addition in two men of 91, and one of 101 years. The index declines 0.18 liter (7.3 per cent) during this period or in each decade ~~0.35~~ 0.235 liter (1.5 per cent). The correlation is negative and is -0.1862 ± 0.0965. It is not significant. But if the entire series of 103 men is utilized it is -0.2039 ± 0.0944 and is significant. The rate of decline is almost the same.

2. The mean values in succeeding decades or half-decades suggest that the rate of decline of the curve is not straight but is arrested for a time after 55 years. To smooth the curve more observations are required. Variations in each age group exist as in younger men. In any 5 or 10 year group the maximum deviations from the respective means were 21.6 and 27.1 per cent. The coefficient of variation of the means varied from 8.3 to 16.6 per cent.

3. For the entire series (40 to 101 years) the mean is 2.26 liters per square meter per minute; the standard deviation is 0.26 (11.6 per cent of the mean) and the standard error of the mean is 0.026. For the evenly

distributed group of 100 men the results are unchanged; the mean value was 2.26 liters, the standard deviation 0.26 (11.5 per cent of the mean), the standard error of the mean 0.026, and the standard error of estimate 0.259 liter.

4. The decline in cardiac index follows chiefly upon the decline of the oxygen consumption since the influence of change in arterio-venous oxygen difference is relatively small.

5. Though the measurement of the index indicates slight decrease in the work of the heart under basal conditions, there remain to be considered other functions which are involved in changes in cardiac activity with age.

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RENAL FUNCTION IN NORMAL AND ADRENALECTOMIZED OPOSSUMS AND EFFECTS OF POST-PITUITARY AND CORTICO-ADRENAL EXTRACTS[1]

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The adrenalectomized opossum, unlike other animals (cat, rat, dog) suffering from adrenal insufficiency, shows no diminution but rather a rise in serum sodium and chloride levels following operation (Silvette and Britton, 1936). It has been suggested that this is a species difference dependent upon a type of kidney which responds to adrenalectomy by a diminished rather than an increased excretion of sodium chloride. The following experiments were designed to test this hypothesis and also to shed light on the effect of ingested salt and water and of injected post-pituitary and cortico-adrenal hormones on renal excretion in this animal type. Female opossums were observed before and after operation in all cases. A preliminary fasting period of 24 hours was observed. The chemical and operative procedures used have been previously described (Silvette and Britton, 1936).

RESULTS. When normal opossums received by mouth 100 cc. per kilo body weight of 1 per cent sodium chloride solution, they excreted in 24 hours about 60 per cent of the ingested water and 85 per cent of ingested salt (table 1, A). The same animals under similar conditions were subsequently injected with 1 cc. of post-pituitary solution (Squibb)[3] and their responses noted. It was observed that the hormonal treatment caused an increase in both the rate and absolute quantity of urine excreted, starting very shortly after injection. Almost 75 per cent of the ingested water was eliminated, and about 95 per cent of the ingested salt (table 1, B). The animals were then adrenalectomized, and when early adrenal insufficiency developed (as evidenced by weight loss), they were placed once more in metabolism cages. A striking inability to excrete ingested salt and water was noted: less than 30 per cent of the ingested water and

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[2] E. R. Squibb and Sons Scientific Fellow in Physiology.

[3] Containing 5 International units per cubic centimeter. Furnished through the kindness of Dr. John F. Anderson of E. R. Squibb and Sons.

correspondingly small quantities of salt were eliminated (table 1, C). When such adrenalectomized animals were given post-pituitary liquid in addition to sodium chloride solution, however, a restoration of normal urinary excretion, both in respect to volume and composition, was observed (table 1, D).

It may be noted from table 1 that the concentration of both sodium and chloride per cubic centimeter of urine did not differ appreciably throughout the various experiments. The volume of water excreted, however, varied remarkably, and so influenced the total daily sodium chloride output. With respect to urea excretion, however, the situation was different. The amount of urea excreted per cubic centimeter of urine

TABLE 1

Effect of post-pituitary liquid on the renal secretion of normal and adrenalectomized opossums

SERIES*	NUMBER OF ANIMALS IN SERIES	URINE VOLUME	URINARY EXCRETION PER KILO BODY WEIGHT					
			Sodium		Chlorides		Urea	
			Per cc.	Total	Per cc.	Total	Per cc.	Total
		cc./kgm	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A. Normal, P. E	5	61±4.6	5.31	324± 5.4	8.85	540± 8.2	7.45	455±40
B. Normal, pituitary-injected, P.E.	5	73±5.8	5.27	385±20.2	7.85	573±23.1	6.09	445±41
C. Adrenalectomized, P.E.	5	28±4.0	4.61	129±22.5	7.96	223±50.4	12.0	336±31
D. Adrenalectomized, pituitary injected, P.E.	6	65±5.1	4.74	308±15.8	7.82	507±24.2	7.42	481±36

* All animals given 100 cc. 1 per cent NaCl solution per kilo body weight per day. Metabolic period, 24 hours.

varied inversely with the fluid output, so that daily urea nitrogen excretion was fairly constant under all conditions.

Post-pituitary liquid was observed to increase the rate of urine excretion in the opossum. Normal animals kept in metabolism cages for three successive days were fed 100 cc. of 1 per cent sodium chloride solution per kilo body weight per day. They excreted, on the average, 49 cc. of urine the first day, 94 cc. the second, and 72 cc. on the third (table 2, A). A series of adrenalectomized animals under the same conditions excreted 35 cc. of urine on the first day, 71 on the second and 99 on the third (table 2, B). The total 72-hour excretion in the two series was, therefore, almost the same—215 cc. and 205 cc. respectively. Apparently in the absence of the adrenal glands the opossum kidney does not excrete ingested fluid at the normally rapid rate, for during the first day an oliguria supervenes

while diuresis does not occur until the third 24-hour period. When adrenalectomized animals were given post-pituitary solution along with sodium chloride solution, a return to the normal condition was observed, i.e., diuresis occurred on the second day (table 2, C). From these experiments it appeared that the difference between a, normal; b, adrenalectomized untreated, and c, adrenalectomized post-pituitary-treated opossums in 24-hour metabolism tests are those involving rate of urine secretion.

TABLE 2

Effect of adrenalectomy and post-pituitary solution on the rate of water and salt elimination in the opossum

SERIES*	NUMBER OF ANIMALS IN SERIES	DAY IN METABOLISM CAGE	AVERAGE DAILY URINARY EXCRETION PER KILO BODY WEIGHT				
			Volume	Sodium		Chlorides	
				Per cc.	Total	Per cc.	Total
			cc.	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A. Normal	4	1st	49	5.19	254	9.02	442
		2nd	94	3.94	371	6.50	611
		3rd	72	4.68	337	7.62	549
		Average	72	4.46	321	7.40	534
B. Adrenalectomized	5	1st	35	4.40	154	6.95	243
		2nd	71	4.17	296	6.67	473
		3rd	99	3.62	358	5.84	579
		Average	68	3.99	269	6.35	432
C. Adrenalectomized, pituitary-injected	2	1st	30	5.97	179	9.55	286
		2nd	107	4.35	465	6.43	688
		3rd	93	3.19	297	5.61	522
		Average	77	4.14	314	6.56	499

* All animals given 100 cc. 1 per cent NaCl solution by mouth per kilo bodyweight per day; urine collected at end of 24, 48 and 72 hours.

In order to determine whether the responses observed in the above experiments held true for different salt concentrations, hypotonic sodium chloride (0.1 per cent) was employed in another series of animals kept in metabolism cages for 24-hour periods. Normal opossums were given orally 100 cc. per kilo body weight of this hypotonic solution per day. They were found to excrete on the average 77 per cent of the ingested water and more than 80 per cent of the salt (table 3, A). Adrenalectomized animals excreted 82 per cent of the water, all of the orally-admin-

istered sodium chloride, and an appreciable amount of the tissue salts as well (table 3, B). Thus it appears that when hypertonic salt solution is ingested, the adrenalectomized opossum does not eliminate excess salt (see preceding paragraph and table 1, C); when in contrast hypotonic saline is ingested, the animal is apparently unable to, or at least does not, prevent sodium chloride loss.

Adrenalectomized opossums given simultaneously 0.1 per cent sodium chloride solution and post-pituitary liquid excreted approximately twice as much sodium and chloride as did unoperated untreated controls (table 3, C). In contrast, adrenalectomized animals given saline solution together with cortico-adrenal extract excreted much less salt than did the

TABLE 3

Effect of cortico-adrenal extract and post-pituitary solution on renal secretion in the opossum

SERIES*	NUMBER OF ANIMALS IN SERIES	URINE VOLUME	URINARY EXCRETION PER KILO BODY WEIGHT					
			Sodium		Chlorides		Urea	
			Per cc.	Total	Per cc.	Total	Per cc.	Total
		cc./kgm.	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*
A. Normal, P. E.	7	77±5.1	0.38	29± 5.1	0.68	52± 7.2	7.05	543±32
B. Adrenalectomized, P.E.	9	82±5.4	0.54	44± 4.6	0.91	75± 9.1	7.28	596±45
C. Adrenalectomized, pituitary-injected, P.E.	5	92±7.9	0.70	64±14.3	1.03	95±17.0	7.68	706±92
D. Adrenalectomized, cortico-adrenal extract, P.E.	7	80±4.0	0.26	21± 4.1	0.49	39± 6.8	9.10	727±34

* All animals given 100 cc. 0.1 per cent NaCl solution by mouth per kilo body weight per day; metabolic period 24 hours.

untreated adrenalectomized animals (table 3, D). In fact, from the standpoint of both volume and inorganic composition, the urine of these animals was very similar to that of the unoperated controls. Cortico-adrenal extract administration led to the excretion of a relatively large amount of urea, for although the fluid output remained almost stationary, the concentration of urea per cubic centimeter of urine rose from the control level of 7.05 mgm. to 9.10 mgm. per cc.

Discussion. According to recent findings, injection of post-pituitary solution is followed by a brief diuresis and then a more lasting antidiuretic effect. No antidiuretic phase was observed, however, after injection of such solutions into the opossum. At first glance it would seem that, since administration of post-pituitary extract to adrenalectomized animals

fed 1 per cent sodium chloride solution leads to an increase in (or a return to normal) renal excretion, the case is one of true replacement therapy. But adrenalectomized opossums given 0.1 per cent saline lose more salt than normal animals under similar conditions, and when simultaneously treated with pituitary solution, they lose even greater amounts. Such hormonal injections therefore appear to have only a coincidental beneficial effect; when conditions happen to be favorable, they may overcome the renal insufficiency which follows upon adrenal removal. The effect of cortico-adrenal extract on salt excretion in the adrenalectomized animal is, in contrast, a specific one. When sodium chloride loss is abnormal, injection of adrenal extract leads to diminished salt excretion and a resultant saving of sodium chloride in the organism.

Administration of post-pituitary solution to adrenalectomized animals had no effect on urinary nitrogen excretion as evidenced by the urea output. In no experiment did this material increase the amount of urea excreted per cubic centimeter of urine. On the other hand, injection of cortico-adrenal extract led to a marked unit-volume increase in urea eliminated.

The total amount of urea excreted by normal or adrenalectomized animals, with or without pituitary liquid, is independent of the fluid volume. The amount of sodium chloride excreted is, on the contrary, a direct function of the urine volume. The difference observed in the excretion of salt and of urea is suggestive. It would indicate that the adrenalectomy does not affect all the functions of the kidney. Urea excretion is apparently unaffected by the absence of the adrenal hormone. And since the amount of salt excreted by the adrenalectomized salt-fed opossum varies directly with the water output, the renal deficiency would seem to be limited to a partial inability to excrete, or a retention of, water. The urea concentrations observed lead to the belief that glomerular filtration is unaffected. An increased tubular reabsorption of water would thus appear to occur in the absence of the adrenal glands.

SUMMARY

Adrenalectomized opossums given 1 per cent NaCl solution by mouth show a remarkable inability to excrete the ingested salt and water. Within 24 hours only 35 per cent of the Na and Cl were eliminated, in contrast to 85 per cent excretion by the normal controls. The concentrations of Na and Cl per cubic centimeter of urine did not differ significantly, however, from conditions observed in the normal animal.

Administration of post-pituitary solution to adrenalectomized opossums under the same conditions led to considerable increases in water and salt excretion, even beyond the amounts eliminated by normal animals. Post-pituitary solution also caused increased salt and water output in normal animals.

The total daily urea excretion was approximately similar in all the foregoing series. The concentration of urea per cubic centimeter of urine varied inversely with the quantity of fluid excreted.

When adrenalectomized opossums were given 0.1 per cent NaCl solution by mouth they excreted more water and salt than normal animals under the same conditions. Administration of post-pituitary extract to such adrenalectomized animals led to a *further* increase in water, Na and Cl excretion.

The injection of cortico-adrenal extract to adrenalectomized opossums given hypotonic (0.1 per cent) NaCl solution resulted in marked reduction in Na and Cl excretion. In comparison, it may be stated, untreated adrenalectomized opossums lost through the kidneys about 100 per cent more NaCl than the extract-treated animals.

It would appear from the results that post-pituitary injections may lead under some conditions to a detrimental loss of Na and Cl from the body. In contrast, cortico-adrenal extract appeared beneficial in adrenal insufficiency by conserving sodium and chloride at normal values.

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THE RATE OF DISAPPEARANCE OF INTRAVENOUSLY ADMINISTERED DEXTROSE IN THE HUMAN SUBJECT

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The purpose of this investigation was not to determine by what mechanism dextrose is metabolized, but rather to ascertain the quantitative relationship of its net loss from blood stream to the total amount present in the circulation. The rate of its disappearance might then be an index as to whether it was stored or utilized through metabolism as measured by respiratory quotients. The net amount of dextrose in circulation was measured by determining the plasma volume and the amount of dextrose as grams per cent. In measuring the plasma volume we used the method developed by Gibson and Evans (1937) in which the plasma volume is determined by the introduction of a blue-azo dye (Evans Blue, Eastman Kodak Co.), and the red cell volume measured by proportion of plasma to red cells (hematocrit). Blood sugar determinations on plasma were carried out by the colorimetric ferricyanide method of Folin, in which the color measurements were carried out by the use of the Evelyn colorimeter (1936). Occasional associated studies were made on the blood lactic acid and the inorganic phosphates.

The following experiment which is described in detail was confirmed in four other normal human subjects. The subject (M.P.) was placed at complete rest in the supine position for 1 hour. During the entire procedure and immediately following the experiment, all the urine was collected. Following the one hour of rest several basal respiratory quotients were determined by collecting samples of expired air in a Tissot spirometer with an aliquot sampling device attached. The determinations of oxygen and carbon dioxide were carried out by the use of Haldane-Boothby apparatus. The usual precaution was taken by checking the apparatus before each experiment by performing an analysis of outdoor air. Following two or three basal determinations on the subject, a sample of blood was withdrawn from a large vein in the antecubital fossa and studies were made from this sample of blood. Subsequent to this 10 cc. of a 2 per cent solution (10 mgm.) of dye (Evans' blue) was administered and the blood volume determined. On each sample of blood withdrawn repeated stud-

ies on the blood chemistry were carried out. Fifty cubic centimeters of a 50 per cent solution of dextrose (25 grams) were then given intravenously.

To circumvent effects due to emotional disturbances from repeated venipunctures only one puncture was made and the lumen of the needle kept unobstructed by a flow of 1 cc. saline per minute. By the use of dry syringes repeated samples for blood volume and blood sugar determinations were taken every 30 seconds for 2 minutes and subsequently every 5 to 10 minutes for a period of ½ hour following the injection of dextrose. By this method we could calculate the amount of dextrose present in the circulating plasma volume. The results are presented in table 1.

TABLE 1

Disappearance of intravenously administered dextrose from the circulating plasma
50 cc. of a 50 per cent solution (25 grams)

TIME	TIME AFTER INJECTION	PLASMA VOLUME	PLASMA SUGAR	AMOUNT PRESENT IN CIRCULATING PLASMA	TOTAL AMOUNT WITHDRAWN FROM PLASMA	PER CENT REMOVED FROM PLASMA	RESPIRATORY QUOTIENT
		cc.	*grams per cent*	*grams*	*grams*		
8:45 Basal		3,170	0.10	3.17	0	0	0.77
8:50–8:53 Dextrose, 25 grams (intravenously)							
8:54	1	3,293	0.25	8.22	19.95	87.5	0.77
8:55	2	3,305	0.25	8.31	19.95	87.5	0.75
8:56	3	3,287	0.25	8.22	19.95	87.5	0.78
8:57	4	3,215	0.22	7.08	21.09	88.13	0.78
8:58	5	3,201	0.22	7.06	21.11	88.15	0.78
9:10	17	3,219	0.19	6.03	22.14	88.74	0.81
9:18	25	3,024	0.166	5.08	23.09	89.17	0.81
9:30	37	2,987	0.165	4.92	23.25	89.25	0.79

Total urine output during course of experiment was 280 cc. and contained 1.8 grams of dextrose.

From the table it can be seen that 87.5 per cent of the added dextrose was removed from the circulating plasma within 4 minutes from the time of injection and that 89.25 per cent had been withdrawn at the end of 36 minutes. There was no rise in the respiratory quotient of any significance until 36 minutes had elapsed. We were particularly struck by the rapid removal of dextrose from the circulation after such an excess had been given intravenously. Since experiments of Soskin and his co-workers (1935) show that dextrose was primarily stored in the liver and secondarily in the other body tissues, with some of the dextrose possibly entering the red cells, the capacity of the organism to store this carbohydrate is a highly efficient one. The failure of the respiratory quotient to rise significantly would point to this view, and as there was an increase in blood lactates the slight change in respiratory quotient cannot necessarily be ascribed to

the metabolism of dextrose. The depression of the inorganic phosphates following the administration of dextrose previously noted by numerous investigators is of interest as it is probably associated with glycogenesis.[1] The slight increase in plasma volume (4 per cent from the basal value) following intravenous dextrose and its subsequent dimunition (5.6 per cent from the basal value) may be the result of changes in viscosity due to an increased concentration of this carbohydrate.

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[1] In order not to detract from the purpose of this communication we have purposely deleted the associated biochemical changes.

THE INFLUENCE OF ADRENAL CORTICAL HORMONE PREPARATIONS ON THE SPONTANEOUS ACTIVITY OF THE NORMAL ALBINO RAT

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Reports by Eagle, Britton and Klein (1932), Indovina (1933) and Baena (1934) that adrenal cortical hormone administration to animals, forced to run, increases the time required for complete exhaustion and thus enables performance of more work, and that by Hitchcock and Grubbs (1937) showing an increase in the efficiency of muscular work in man following administration of this hormone, have aroused interest in the possibility of demonstrating other effects on the neuromuscular functions of normal animals. The spontaneous running of rats in rotating cages suggested itself for study, particularly since Durrant (1924) and Richter (1936) have found this activity to be markedly reduced by complete adrenalectomy, this effect being due to a loss of cortical and not medullary tissue according to Ingle and Harris (1936). Richter was able to restore partially the normal activity level by the oral administration of Grollman's charcoal adsorbate of the adrenal cortical hormone. The present paper presents the results of administering this and other preparations of the hormone on the spontaneous activity of the unoperated albino rat.

METHODS. Albino rats of the Slonaker-Wistar strain were used, their ages at the beginning of the experiment being 207 to 273 days. The same animals, except for a few replacements, were used throughout the investigation which lasted from August 1936 to April 1937.

Spontaneous activity was determined by means of the rotating cages described by Slonaker (1907 and 1908), the cyclometers being read daily at the same hour. The cages containing the females were spaced far enough away from those containing the males to prevent estrous phenomena influencing the activity of the latter (Slonaker, 1935). A dry diet, based on corn meal (70.9 per cent), with addition of linseed oil cake (9.7 per cent), alfalfa meal (1.9 per cent), casein (9.7 per cent), biotol (4.9 per cent), bone ash (1.4 per cent), sodium chloride (0.5 per cent) and wheat

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germ (1 per cent) was available to the animals for about three hours each morning. The sodium content of this diet was 0.37 per cent, the potassium content, 0.40 per cent. Water was constantly available to the animals.

Three preparations of adrenal cortical hormone were administered in successive experiments:

1. A Grollman charcoal adsorbate, prepared by the Merck Institute for Therapeutic Research. The material was intimately mixed in a mortar with finely ground liverwurst and weighed aliquots given to the rats at the end of their 21-hour fast. The animals always took the masses promptly. We are thus assured that each animal actually ingested the amount of adsorbate assigned to it each day. A dose of 0.16 gram per rat per day was given in the first experiment, a dose of 0.32 gram per rat per day in the second. Control animals received in liverwurst a quantity of the charcoal adsorbent (Norit) equal to that given to the treated animals.

2. Eschatin, a Swingle-Pfiffner extract prepared by Parke Davis and Company. Two cubic centimeters were injected intraperitoneally daily. Each control received 2 cc. 0.5 per cent phenol (the preservative of the Eschatin) in 0.9 per cent NaCl daily.

3. The Adrenal Cortex Extract prepared by the Wilson Laboratories. One cubic centimeter of this material was injected intraperitoneally daily. Controls received 1 cc. 1:100,000 merthiolate (the preservative of this extract) in 0.9 per cent NaCl daily.

The three preparations were subjected to bioassay on a single strain of rats following the method of Grollman (1936), but using a low salt diet. On the assumption that the quantity of a preparation yielding a mean weight increase of 1.5 grams per rat per day contained $\frac{1}{2}$ rat unit, the potencies of the preparations were: Grollman adsorbate, 3.18 rat units per gram; Eschatin, 0.16 rat unit per cubic centimeter; Wilson's Extract, 0.40 rat unit per cubic centimeter.

In each experiment, equal numbers of rats received the hormone preparation and control material. All available animals were arranged in order of activity during the pre-administration control period; the odd-numbered rats were made controls and the even-numbered rats received the preparations. Thus, each group contained as far as possible equal representation of very active, moderately active and relatively inactive individuals.

Results. In table 1 are presented the number and sex of treated and control animals in each experiment, the dose of hormone preparation administered, and the average activity level (in revolutions per rat per day) in each period. In table 2 are given the durations of the pre-administration control, the administration and post-administration periods. During the first two experiments, those on the charcoal adsorbate, the general activity level tends to decrease progressively in all groups of animals.

TABLE 1

Effect of adrenal cortical hormone preparations on spontaneous activity

RATS		PRE-ADMINISTRATION PERIOD	ADMINISTRATION PERIOD		POST-ADMINISTRATION PERIOD	
		Activity	Activity	Change from pre-administration	Activity	Change from pre-administration
Grollman adsorbate (0.5 rat unit per day)						
		rev. per day	*rev. per day*	*per cent*	*rev. per day*	*per cent*
Males:						
Controls	6	4,477	4,068	−9.1	3,251	−29.4
Treated	6	4,075	3,342	−18.0	2,878	−29.4
Females:						
Controls	5	7,881	7,279	−7.6	5,845	−25.8
Treated	5	6,802	6,332	−6.9	5,492	−19.3
Grollman adsorbate (1.0 rat unit per day)						
Males:						
Controls	10	4,614	3,039	−34.1	2,531	−45.1
Treated	10	4,112	2,945	−30.0	2,198	−46.5
Females:						
Controls	5	4,619	2,931	−36.5	2,546	−44.9
Treated	4	4,637	1,817	−60.8	2,096	−54.8
Eschatin (0.32 rat unit per day)						
Males:						
Controls	10	2,401	2,103	−12.4	2,067	−13.9
Treated	10	2,160	2,187	+1.2	2,411	+11.6
Females:						
Controls	5	2,624	2,216	−15.5	3,047	+16.1
Treated	4	2,136	1,993	−6.7	2,249	+5.3
Wilson's extract (0.40 rat unit per day)						
Males:						
Controls	10	2,348	2,527	+7.6	2,810	+19.7
Treated	10	2,130	1,971	−7.5	2,365	+11.0

TABLE 2

Duration of periods in experiments

PREPARATION	PRE-ADMINISTRATION PERIOD	ADMINISTRATION PERIOD	POST-ADMINISTRATION PERIOD
	days	*days*	*days*
Grollman adsorbate (0.5 rat unit per day)	16	19	10
Grollman adsorbate (1.0 rat unit per day)	22	21	22
Eschatin	18	15	15
Wilson's extract	23	20	17

This is characteristic of rats in this period of life and is unrelated to the administration of our hormone and control preparations. The activity remains practically constant during the latter two experiments.

The results of all the experiments may be summarized by the statement that no statistically valid differences between the effects of the adrenal cortical hormone preparations and of the control preparations on activity were found. Such differences as did occur were about equally divided between increases and decreases in the treated animals as compared with their respective controls. However, the variability in performance of the animals in any group is so great as to prevent slight effects from appearing. Thus, the apparent decrease in activity produced by 1 cc. of Wilson's extract, as shown by a 7.5 per cent fall in mean activity as compared with an increase of 7.6 per cent in the controls, is one which could have occurred 45 times in 100 in random sampling of a population in which no such factor was acting (Fisher's method for small samples). In the post-administration period, no statistically valid effects of the administration of these products were manifested.

It may be concluded that the administration of these preparations of the hormone of the adrenal cortex in doses of 0.32 to 1.0 rat unit per day failed to exert any effect on spontaneous activity sufficiently marked to be demonstrable by a technique with as great inherent variability as that here employed.

Discussion. The question may be raised as to whether the doses of hormone preparations which we have given actually raised the concentration of the hormone in the body fluids above normal. Apart from the probability of a greater rate of destruction and excretion under the conditions of administration employed, it is possible that the rate at which the animals' own adrenal glands secrete the hormone is decreased when additional quantities are provided from external sources. Thus, thyroxine is known to cause atrophy of the normal thyroid gland (Gray, Haven and Loeb, 1927). To be certain of achieving an abnormally high concentration of the hormone in the body fluids, one would have to administer quantities greater than the gland secretes normally. This is probably about 3.5 rat units per day, the amount found by Grollman (1936) adequate to maintain an adrenalectomized adult. A comparison of our doses with this requirement is made difficult by our use of a low salt diet in assay, a procedure which increases the size of the unit. Even so, they doubtless fall considerably short of the quantities needed for certain hormonal excess.

It has been frequently emphasized that the adrenal cortical hormone's action is most evident under conditions involving induction of fatigue or other unfavorable states. Thus, Hitchcock and Grubbs (1937) report that while the hormone has little effect on basal oxygen consumption, it increases the efficiency of muscular work in man in greater degree as the

work is made more strenuous. In contrast to conditions in which work is imposed and fatigue develops, the spontaneous activity of an animal in a rotating drum, in which the running is not motivated by success in finding food or other reward, is a self-limiting function. Presumably, as soon as any aspect of central nervous system activity on which running depends becomes significantly depressed, that running stops. The absence of serious departures from normal in the animal's economy renders manifestation of the corrective action of the adrenal cortical hormone impossible.

In view of these considerations, we do not consider the negative findings herein reported as conflicting in any sense with the positive reports of those workers who have observed beneficial effects under conditions of imposed vigorous exercise.

SUMMARY

The spontaneous activity of normal albino rats in rotating cages is not significantly altered by administration of moderately large doses of various preparations of the hormone of the adrenal cortex over periods of two to three weeks. The self-limiting nature of this form of exercise prevents the development of fatigue, a condition apparently necessary to the exhibition of the beneficial influences of the hormone in general bodily activity.

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THE EFFECT OF ADRENAL CORTICAL EXTRACT UPON THE OXYGEN CONSUMPTION OF NORMAL HUMAN BEINGS[1]

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The fact that the adrenal cortex influences respiratory metabolism is well established. Among the investigators who have reported a lowered respiratory metabolism in double adrenalectomized animals may be mention Aub, Forman and Bright (1922); Kaiwa (1932); Webster, Pfiffner and Swingle (1932); and Harrop, Weinstein, Soffer and Trescher (1933). The two groups of investigators last mentioned have also shown that the lowered oxygen consumption which follows double adrenalectomy can be restored to normal by the administration of adrenal cortical extract. Koehler and Hastings (1929) reported that adrenalin free extracts given orally raised the metabolism of dogs and mice and exerted a calorigenic effect upon human subjects who had a low metabolic rate. On the other hand Franke (1932) found that subcutaneous injections of cortical extracts caused a diminution of the basal metabolism of dogs. Favorable effects of cortical extracts upon work capacity have been reported by Eagle, Britton and Kline (1932); Barilari (1933); and Ingle (1936).

METHOD. We have investigated the effects of the intravenous or subcutaneous injection of large doses of adrenal cortical extract on the oxygen consumption of normal human beings. The respiratory metabolism of the various subjects was measured by the Tissot-Haldane technique *1*, under basal conditions; *2*, while the subjects were standing erect for a five minute period, and *3*, while they were walking on a treadmill at the rate of 100 meters per minute for a ten minute period. Walking at this rate raises the oxygen consumption to a level of from four to five times the basal requirement and is therefore at the lower limit of the zone which Dill (1936) has designated as hard work.

The amount of extract given the various subjects varied from 1 to 4 cc. (1 cc. equals 10 to 20 C.U.). In control tests injections of normal saline were usually given. Two series of experiments have been carried out using twelve different subjects upon whom a total of more than sixty tests were run.

RESULTS AND DISCUSSION. The first series of experiments were carried

[1] Aided by a grant from the Rockefeller Foundation.

out on eight subjects (five males and three females). The effects of cortical extract upon the basal oxygen consumption of this group are shown in table 1. Each subject had been receiving a subcutaneous injection of 0.5 cc. of the extract twice daily for three or four days preceding the tests, the results of which are tabulated under the heading "During extract." The results which are tabulated under the heading "After extract" were obtained in tests that were run at least a month after the administration of extract had been discontinued. Six of the eight subjects showed a lower basal oxygen consumption during the administration of extract than either before or after. However the differences are very small, the average for the group being only about 1 per cent lower during the administration of extract than for control tests. This is scarcely large enough to be considered significant.

TABLE 1

The effect of adrenal cortical extract on basal O_2 consumption

SUBJECT	SEX	BASAL O_2 CONSUMPTION		
		Before extract	During extract	After extract
		cc. per minute	*cc. per minute*	*cc. per minute*
K. B.	F.	185	180	187
J. D.	M.	239	212	236
D. D.	F.	199	153	153
F. H.	M.	218	215	218
S. H.	M.	230	218	220
M. M.	F.	189	183	185
R. M.	M.	255	238	241
G. T.	M.	248	237	223
Average		214	205	208

The standing and walking tests were run under the same general conditions as the basal tests. The results of the tests run while the subjects were standing erect are shown in table 2. The oxygen consumption is increased about 20 per cent above the basal level. Again six of the eight subjects showed a lowering of the oxygen consumption during the administration of the cortical extract. Both subjects who failed to show this effect were females and with them the effect may have been masked by variations in metabolism that are known to accompany the menstrual cycle. The lowering of the oxygen consumption was of greater magnitude here than in the basal tests. The average for the group was a little more than 10 per cent lower during the period that extract was being administered than in control tests run before and after the extract.

Table 3 gives the oxygen consumption of the same subjects while walking on the treadmill both before and during the administration of the cortical

extract. Due to circumstances beyond our control we found it impossible to conduct a series of control tests after the extract had been discontinued. The oxygen consumption during the administration of extract was lowered in all but one subject, the average difference for the group being about 15 per cent.

TABLE 2

The effect of adrenal cortical extract on the standing O_2 consumption

SUBJECT	SEX	STANDING O_2 CONSUMPTION		
		Before extract	During extract	After extract
		cc. per minute	*cc. per minute*	*cc. per minute*
K. B.	F.	231	213	204
J. D.	M.	273	254	276
D. D.	F.	235	186	253
F. H.	M.	263	251	269
S. H.	M.	271	226	251
M. M.	F.	223	223	213
R. M.	M.	286	263	288
G. T.	M.	318	287	308
Average		263	238	258

TABLE 3

The effect of adrenal cortical extract on O_2 consumption during walking

SUBJECT	SEX	O_2 CONSUMPTION		PER CENT CHANGE
		Before extract	During extract	
		cc. per minute	*cc. per minute*	
K. B.	F.	811	611	−24.7
J. D.	M.	957	715	−25.3
D. D.	F.	944	747	−20.8
F. H.	M.	1,037	924	−10.9
S. H.	M.	977	783	−19.8
M. M.	F.	810	697	−14.0
R. M.	M.	1,148	1,183	+3.0
G. T.	M.	1,031	997	−3.3
Average		964	832	−15.9

The subjects used in this first series of experiments were untrained in such experiments. Most of them had never walked on a treadmill before. Part of the reduction in oxygen consumption, especially during walking, might therefore be the effect of training. In order to control this factor and to determine the effect of variation in the dosage and the duration of the effect after the administration of extract was discontinued, a second

series of experiments was carried out. No females were used as subjects in these experiments since we felt that the variations in metabolism which are known to accompany the menstrual cycle might complicate the results (Benedict and Finn, 1928; Hitchcock and Wardwell, 1929). In this second series of experiments control tests were run at intervals of one week until two determinations were obtained which checked within 3 per cent. Basal and standing tests were run in this series of experiments just as was done in the first series. The results obtained verified the conclusions reached as a result of a consideration of the first series and added nothing new, therefore they are not reported in detail.

The results of the walking tests on the four subjects who were studied most extensively are shown graphically in figure 1. F. H. showed a marked depression of oxygen consumption following the administration of extract. This subject was given 1 cc. of the extract intravenously about four hours preceding the first extract test. Administration of extract was begun two days before the second extract test. One quarter of a cubic centimeter was given subcutaneously twice daily, and finally 1 cc. was given intravenously four hours preceding the test. This procedure resulted in the lowest oxygen consumption ever observed with this subject. Four cubic centimeters of the extract were given intravenously four hours before the last extract test. This massive dose (equal to about 60 C.U.) produced no greater fall in the oxygen consumption than did the 1 cc. dose used in the first extract test. A series of control tests run after the last injection of extract showed a gradual tendency for the oxygen consumption to return to normal although the final test which was run more than three months after the last injection gave a result still well below the figures obtained in the original control tests.

The results obtained with R. H. are in general the same as for F. H. and therefore need no comment. With R. A., however, the extract seems to have increased rather than decreased the oxygen consumption. The results are exactly the opposite to those obtained with F. H. and R. H. The explanation of these atypical results may be the fact that R. A. had received a series of injections several months before these experiments. It is possible that an immunity or some other type of resistance may have been built up in this subject as a result of these previous injections. The experiments on the subject F. B. were designed to test this hypothesis.

The first extract test on this subject shows the effect of the intravenous injection of 1 cc. of an extract of very low cortin content. In spite of the fact that this extract contained an almost negligible amount of the vital hormone there was a noticeable lowering of the oxygen consumption. After two more control tests a series of three extract tests was run on alternate days. One cubic centimeter was given intravenously before the first test and $\frac{1}{2}$ cc. of the regular extract was given subcutaneously twice a day

thereafter. The injections were discontinued about eighteen hours before the third and last extract test of this series. The following week a control test was run and then the next week 1 cc. of the strong extract was given

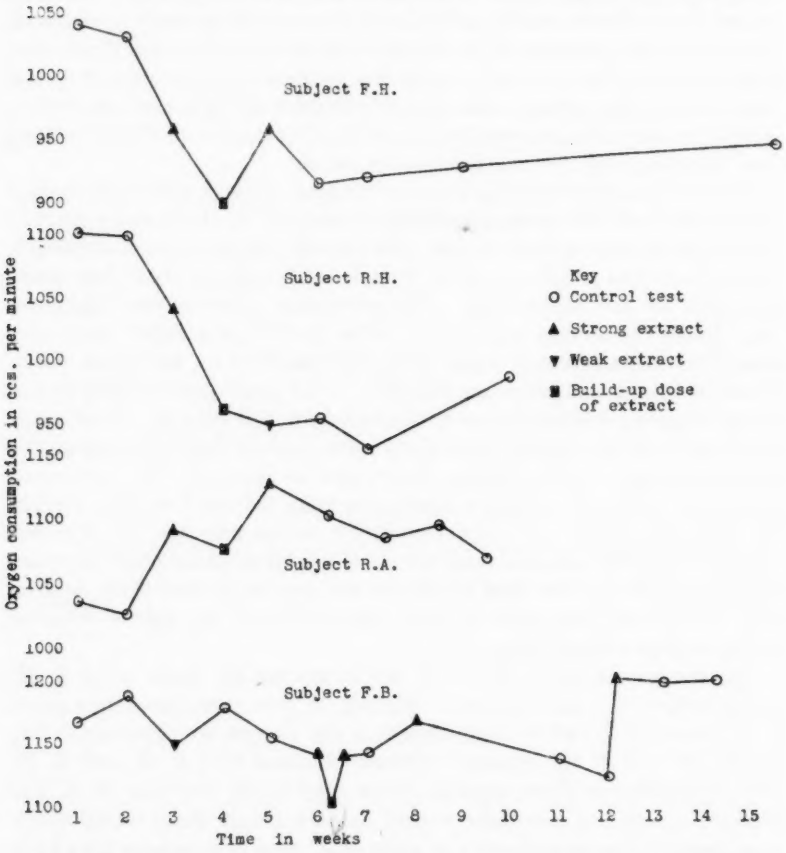

Fig. 1. The effect of adrenal cortical extract on the oxygen consumption of normal human subjects while walking on a treadmill at the rate of 100 meters per minute. Extract was given intravenously four hours before the test. In buildup doses small subcutaneous injections were given twice daily for two days preceding the test with an intravenous injection given four hours before the test.

intravenously. This caused a rise rather than a fall in the oxygen consumption. The same procedure was repeated a month later with an even more pronounced rise in the oxygen consumption. These results seem to indicate that injection of cortical extract causes some physiological reaction

which results in later injections causing a rise rather than a fall in the oxygen consumption while walking.

Hartman, Lewis and Toby (1937) have reported that the effect of cortical extract on electrolyte excretion is diminished or abolished following repeated injection of the extract. This reversal of the effect on oxygen consumption is more significant when considered in the light of their experiments. The effect of the extract on electrolyte excretion was studied in our subjects at the same time the respiratory metabolism was determined. It was found that when the oxygen consumption was reduced there was invariably a retention of sodium and an increased excretion of potassium. However, in all cases where a rise in oxygen consumption followed the administration of extract this effect on electrolyte excretion was much diminished or abolished.

The effect on oxygen consumption may or may not be due to the vital hormone. The fact that the ability of an extract to reduce oxygen consumption while the subject is doing light work does not run parallel to its cortin content, would seem to indicate that we are dealing with some other factor than the vital hormone. However at the present time we can do nothing more than theorize on this point.

Another point that is worthy of comment is that the prolonged after-effect on oxygen consumption noted in these experiments is similar to the after-effect on conditioned reflexes in sheep noted by Liddell, Anderson, Kotyuka and Hartman (1935). This suggests the possibility that we are dealing here indirectly with an effect on the nervous system. Most of our subjects reported subjective effects characterized by greater relaxation and an increased tendency to sleep. Such a condition might very well result in a general decrease in muscle tonus and so cause a decrease in oxygen consumption.

SUMMARY

Experiments on the effect of adrenal cortical extract on the oxygen consumption of normal human beings in which more than sixty tests were carried out on twelve different subjects showed that:

1. The extract produced no significant effect on the basal oxygen consumption.

2. The oxygen consumption while standing erect was significantly reduced in nine out of twelve subjects after the administration of extract. This depression averaged nearly 6 per cent.

3. There was a still greater depression of the oxygen consumption while the subjects were walking on a treadmill at the rate of 100 meters per minute. This occurred in ten of the twelve subjects and averaged about 10 per cent.

4. The effect on the oxygen consumption was not always parallel to

the content of vital hormone of the extract and therefore may be due to some other factor.

5. The effect is both cumulative and persistent and some evidence is presented to show that repeated injections of the extract cause a reversal of the effect.

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THE EFFECTS OF ADRENAL CORTICAL EXTRACT IN REST AND WORK

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The fact that the adrenal cortex has an essential rôle in maintaining skeletal muscles in working order is unquestioned. It is not so certain that the administration of extracts of the cortex to a normal animal modifies its ability and willingness to perform physical work or the efficiency with which the work is performed. The pertinent literature has been reviewed in the foregoing papers by Hall and Müller (1) and by Hitchcock, Grubbs and Hartman (2). Our work has been in collaboration with the latter authors and their cortin preparations have been used exclusively. In addition to studying performance in muscular work of about the intensity used in their experiments, we have employed two higher grades of activity. In one of these the oxygen consumption was about 7 times basal, and in the other the work intensity was such that the highest attainable level of oxygen intake was reached in about 3 minutes; exhaustion usually terminated the experiment within 5 minutes. The nature of the observations made is apparent from the figures; references may be made to earlier publications (3) for description of the methods used.

Four subjects have been employed, all of whom were accustomed to walking and running on our motor-driven treadmill. The subject came to the laboratory in the fasting state, and observations of pulse, blood pressure, basal metabolic rate (B.M.R.), etc., were made before exercise was begun.

In the first series of experiments a weak extract, 2 to 3 cat units per cc., was administered in doses of $\frac{1}{4}$ cc. twice daily to 2 subjects and a strong extract, 40 to 80 units, was administered in the same way to 2 other subjects. Seven injections were given to each in $3\frac{1}{2}$ days. In the second series of experiments, carried out several weeks later, the strong extract was administered at the rate of $\frac{1}{2}$ cc. twice daily to each of the same 4 men.

In the first series of experiments the post-injection B.M.R. seemed to be related to the level before injection. Where the basal oxygen intake

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approached or exceeded the standard, the injection of the extract, whether strong or weak, was followed by a small reduction in oxygen intake (subjects L. E. and J. L.). Two subjects had a low initial B.M.R. Of these, D. B. D. received the strong extract and showed no appreciable change; S. R. received the weak extract and exhibited a rise in B.M.R. The larger doses of strong extract were followed by a slight drop in B.M.R. in 3 subjects after 4 to 9 injections (fig. 1). After the injections were stopped, the basal oxygen intake of the subjects showed no uniform variations.

The easiest work, walking with no grade for 15 minutes at 5.6 km. per hour, raised the oxygen consumption to 4 times the basal value. In 3 cases there was a greater oxygen consumption after 9 injections, ½ cc. each, of the strong extract. The increase was appreciable in relation to the

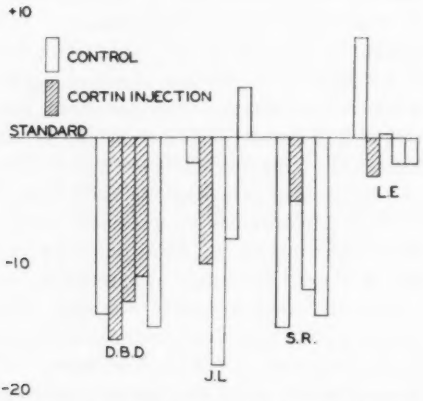

Fig. 1. Basal metabolic rate in relation to the daily injection of 40 to 80 cat units of cortin for 5 days. Observations were continued for 3 weeks after the period of injection.

level of oxygen consumption reached in this grade of work before administration of the hormone. In the post-injection period the oxygen requirement for this work dropped for all our subjects much below the original level but eventually rose nearly to the original level (fig. 2). It is well known that carbohydrate is a more efficient fuel than fat, and it occurred to us that this might have some bearing on the greater efficiency with which easy work is carried on after cortin administration. However, no close correspondence between the oxygen consumption in these experiments and the proportion of energy derived from carbohydrate was revealed by measurements of the R.Q.

The more intensive work, walking at 5.6 km. per hour on an 8.6 per cent grade, raised the oxygen consumption above 1.5 liters per minute. In

both series of injections, whether of strong or weak extract, a slightly decreased oxygen requirement was observed in 3 of the 4 subjects. D. B. D. was an exception; in the first series he showed no change and in the second series, an increase. The most strenuous work, running at 11.2 km. per hour for 4 to 5 minutes, was not rendered easier by cortin administration; in most of the experiments with cortin there was a tendency toward a decreased oxygen intake in the last minutes of work; there certainly was no greater capacity for oxygen intake nor for anaerobic work (fig. 3).

The concentration of lactic acid in blood during work sometimes increased less after cortin. We are not inclined to attach significance to the differences observed, since training can affect the lactic acid level considerably. The fact that lactic acid concentration cannot be raised

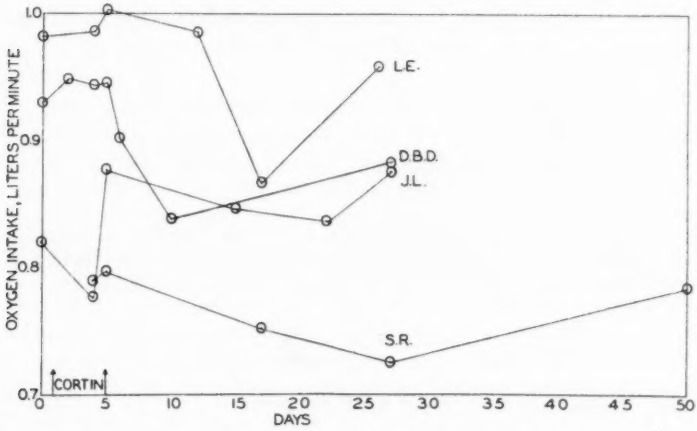

Fig. 2. The oxygen requirement in standardized easy work during and following the injection of 40 to 80 cat units of cortin daily for 5 days.

to a higher level as a result of cortin administration indicates that the capacity for accumulating an oxygen debt is no greater, at least so far as the lactacid mechanism is concerned. No uniform differences in the blood sugar concentration were observed which could be related to cortin in either the moderate or the severe work.

No decided change in number of red cells during rest or work was produced by cortin injections. On the other hand, some rise in the whole number of white cells in rest in two subjects, J. L. and S. R., took place: in subject J. L., 6,500 to 7,150, and in subject S. R., 6,600 to 9,650. These changes arose entirely from an increase in number of neutrophiles: in subject J. L., 2,600 to 3,700, and in subject S. R., 3,830 to 4,730. Increase in number of neutrophiles after cortin administration is similar to the

effect described by Corey and Britton in animals (4). The post-cortin effect on lymphocytes in rest was insignificant and irregular. Increase

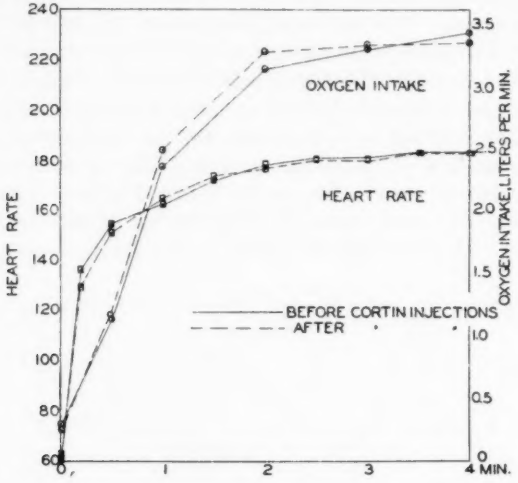

Fig. 3. The rate of oxygen consumption and the heart rate in maximal work of 4 minutes' duration. These experiments were carried out on J. L. before and immediately following the injection of 40 to 80 cat units of cortin daily for 5 days.

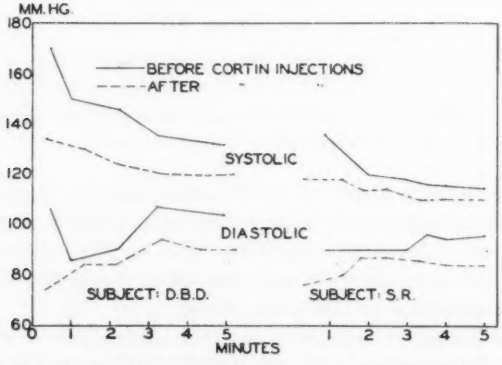

Fig. 4. The blood pressure in recovery from maximal work.

in the whole number of white cells after exercise arose independently of cortin administration from an increase in the number of lymphocytes.

A lower level of blood pressure after the completion of work seemed to be a characteristic effect of cortin administration (fig. 4). In spite of

individual differences in systolic and diastolic pressure, this type of change was found in all cases. The time required for return of the pressure to the initial level was in general shorter after cortin injections. The character of the changes in pulse pressure was the same as before injection.

The heart rate during work recorded by the cardiotachometer was quite similar to the heart rate before cortin administration. The rates for corresponding times during work were nearly identical (fig. 3).

Our data on functional changes and efficiency during work do not allow us to draw any very far-reaching conclusions about the influence of cortin on the processes of muscular activity. None of the subjects reported subjective symptoms either in rest or in work. The objective differences shown by our subjects in their reactions to cortin may be related to the individual balance of nervous and humoral regulation. Future research on the rôle of the adrenal cortical hormone in physiological activity will probably have to pay considerable attention to the functional constitution of the subjects used.

We consider that the small irregular changes observed in B.M.R. generally confirm the latest observations of Hitchcock and Grubbs (5) on men, as well as the results of Riddle, Dotti and Smith (6) on pigeons; in no case have significant changes been found in basal metabolism.

The effect of cortin administration which appeared during muscular work in our experiments was less striking than the report of Eagle, Britton and Kline (7) that there may be an increase in the capacity of trained dogs for work of long duration of over 100 per cent. The decrease in oxygen intake during easy work in the post-injection period was relatively smaller and occurred at a later time than in the experiments of Hitchcock and Grubbs (5). A slightly increased oxygen consumption in easy work during the injection period preceded a decrease in the post-injection period (fig. 2). After a more or less pronounced decrease in oxygen consumption during easy work which lasted, depending upon the subject, from 5 to 22 days after the injections, the oxygen requirement of easy work rose to the initial level.

While the time course of the effects of cortin administration differs from that reported by Hitchcock and Grubbs, we are inclined to attribute this to differences in the concentration and amount of hormone administered. Our results in moderate exercise may be considered as qualitatively like those of Hitchcock and Grubbs (5), Brack (9) and Gaarenstroom, Waterman and Laqueur (10).

We have no evidence to decide whether the mechanism of this effect of cortin is related to some change in circulation, metabolism, or nervous regulation, or is influenced, as supposed by Hartman, by local changes in the muscles. More economical use of carbohydrate reserves or increase of efficiency of the metabolism in muscles has been suggested by Indovina

(11), but our measurements of R.Q. show no greater preferential utilization of carbohydrate after cortin.

A more intensive study, including the determination of oxygen debt in relation to changes in oxygen consumption and lactic acid level in blood, may facilitate interpretation of the rôle of the adrenal cortical hormone in muscular activity. A more detailed analysis of the relation of decrease in blood pressure after work to the well-known influence of cortin on the regulation of the systemic blood volume (12, 13) may be instructive. Finally, a comparison of a single massive dose with prolonged administration should be made in view of the report by Bauer, Kunewälder and Schächter (14) that antihormones are sometimes produced after prolonged injection of certain hormones.

SUMMARY

The injection of $\frac{1}{2}$ to 1 cc. daily for 3 to 5 days of Hartman's cortical adrenal extract produced no striking effects in resting subjects. The efficiency with which easy walking was performed was increased for some days after the period of injection. The capacity for anaerobic work was not increased; the only notable effect in moderate or severe work was in the blood pressure during recovery. Without exception after administration of cortin the blood pressure in the early stage of recovery was lower and the resting value was reached sooner.

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THE EFFECT OF ANTERIOR PITUITARY EXTRACT ON THE RESPONSE OF THE DENERVATED HEART TO ADRENINE

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The object of this investigation was to study the effect of the anterior pituitary on the sensitivity of structures to adrenine. Although numerous observations have been made on the relationship of the posterior lobe to adrenine (Trendelenburg, 1920) not many direct observations have been made on the influence of the anterior lobe. The work of Cope and Marks (1934), who studied blood-sugar changes after hypophysectomy, is suggestive of a depressed sympatho-adrenal activity in this state. They observed that although the hyperglycemic response to adrenine was intensified during administration of anterior pituitary extract, its effect on blood sugar was practically abolished by hypophysectomy. These results were not correlated with the thyreotropic action of the anterior lobe. In the present research an attempt has been made to estimate the influence of anterior pituitary extract on the response of a test organ to adrenine and to determine whether its influence, if any, is exerted independently of other glands or is affected by the presence of the thyroid. Experiments have been performed on normal and thyroidectomized animals and the effects of anterior pituitary and thyroid extracts compared.

METHODS. Since in previous experiments (Sawyer and Brown, 1935) the chronically denervated heart proved a satisfactory test organ for the study of sensitivity to adrenine over a long period, it was again employed in the present investigation. Cats were used for all experiments and in each animal the adrenals were first inactivated under ether anesthesia by removal of one gland and denervation and demedullation of the other. At the same time the hepatic nerves were also severed. After several weeks the heart was denervated according to the method of Cannon, Lewis and Britton (1926). During the experiments heart rates were counted and recorded as previously described (Sawyer and Brown, 1935). Injections were made through a needle inserted into the femoral vein, but an improved device was used to keep it from filling with blood while in place. Instead of the rubber cap used in the earlier experiments, the lower end of a discarded 1 cc. tuberculin syringe was fitted into the needle. The

barrel of this syringe was cut off so that it measured about 0.5 cm., and it was beveled internally to receive a small rubber stopper of the type used to close vaccine bottles. By this means the capacity of the needle and stopper was cut down to less than 0.1 cc. The chief advantage of this device was that it formed a rigid system, prevented back flow of blood and completely eliminated blocking of the needle. A number of injections could be made through the rubber stopper during an experiment without disturbing the needle in the vein.

The standard dose of adrenalin generally employed was 0.5 cc., 1:500,000, freshly prepared from the 1:1,000 solution by diluting with normal saline. In a few cases a weaker solution, 1:1,000,000, was used as well. The control response of the heart of each animal to the standard dose was obtained by testing its reaction several times daily for a period of five days or longer.

Anterior pituitary extract was supplied for these experiments through the courtesy of E. R. Squibb & Sons. It is subsequently referred to throughout this paper as APE.

The thyroid preparations employed were a semi-purified thyroxin (E. R. Squibb and Sons) and a desiccated thyroid (Parke, Davis).

RESULTS. Previous observations revealed that the response of the denervated heart of an individual animal to adrenine was quite constant from day to day, provided the animal was in a fasting condition and not excited at the time of injection (Sawyer and Brown, 1935). In these earlier experiments, however, it was considered necessary to wait from 10 to 14 days after denervation before beginning the control injections, in order to allow time for sensitization of the heart to take place. In the present investigation, in which as long a time as possible was desired for observations before risking regeneration of cardiac vagal fibers, it was found unnecessary to wait that length of time for the heart to become sensitized. The first control injections in some cases were made a few hours after denervation, as soon as the animal was out of ether, and in others within a day or two after the operation. The responses were tested thereafter over a period of 10 to 14 days. It was observed that the rise in heart rate within a day or two after operation, especially in the first half-minute after the adrenine injection, was approximately as great as that obtained a week or so later. Table 1 shows a typical case. Obviously the heart, if affected by denervation, becomes sensitized rapidly.

In the case of cat 30, described in table 1, as well as in some others, the basal heart rate was higher immediately after the denervation than it was several days or a week later. It has, however, been repeatedly observed in the control injections that the response of the heart to adrenine in a given animal is the same regardless of whether the basal rate varies somewhat from day to day or remains constant. It has also been noted during

a daily experiment, when several injections were made at 10- to 15-minute intervals, that the rise in heart rate was the same after each injection although the basal rate had increased slightly. For these reasons it was considered advisable to express the increases in heart rate as actual increments over the basal rate and not calculate them as percentage rises as had been previously done (Sawyer and Brown, 1935).

The effect of anterior pituitary extract. APE was administered to five animals after the response of each to the standard dose of adrenine had been established (figs. 1, 2, 3, 4 and 5). The extract was administered intraperitoneally, in daily doses of 6 to 8 cc., over a period of 7 to 14 days. During this time the response of the heart to adrenine was tested every few days. In four of the five animals the treatment with APE was followed by an increased sensitivity to adrenine. This increase, however, varied both in its amount and in the time required for it to develop. It

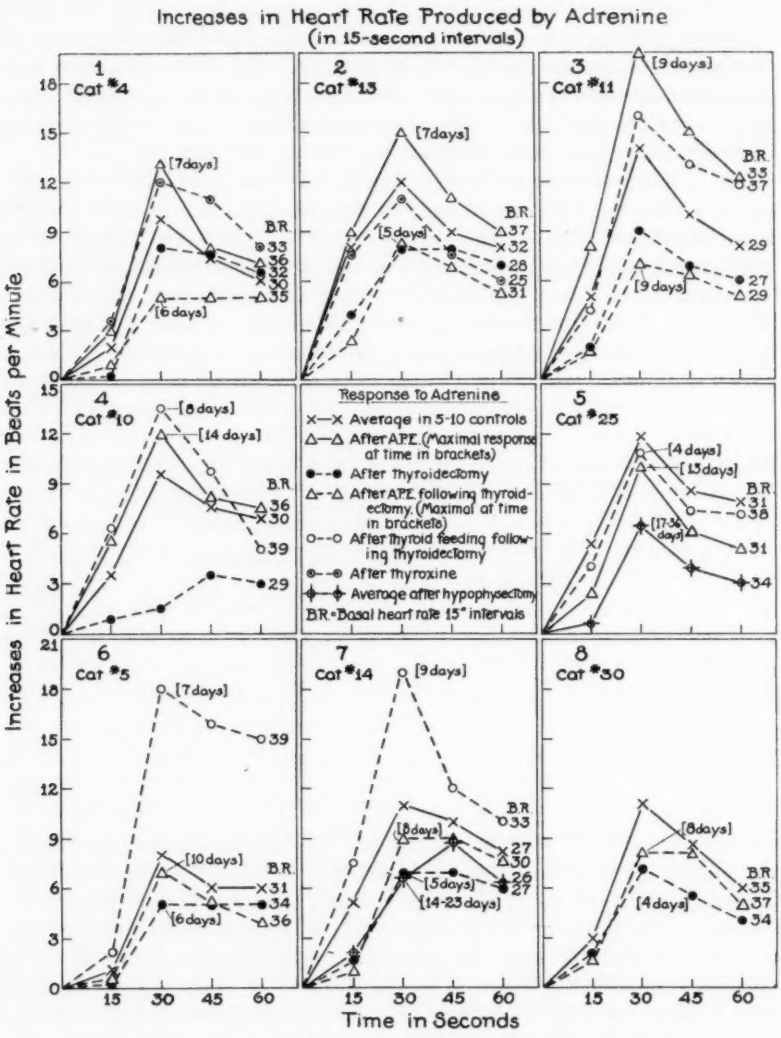
TABLE 1

Response of the denervated heart to intravenous injection of 0.5 cc. of 1:500,000 solution of adrenine. Cat 30. Heart denervation done on April 26

DATE	HEART RATE EVERY 15 SECONDS				
	Basal before injection	Increase over basal in first minute following injection			
		1st quarter	2nd quarter	3rd quarter	4th quarter
April 27	38	3	10	5	5
April 28	40	3	10	8	6
April 29	37	2	10	5	5
April 30	37	3	11	8	8
May 1	35	3	11	9	5
May 3	35	3	11	9	8
May 8	34	3	11	9	9

was not observed in any case during the first few days after the extract was begun. The earliest time after which a definite effect was noted was 5 days (cat 4). In the three other animals (cats 12, 11 and 10) the increase was noted on the 6th, 8th and 10th days respectively. During the next few days in all cases the response showed a further increase. The difference between the increase in the number of beats during the first minute after the injection of adrenine, in the experiments with APE and in the control experiments, is shown in table 2.

The increase in sensitivity to adrenine was greatest in cat 11 (fig. 3). In this animal the maximal rise in the first minute after adrenine was 18 beats greater after 10 days of APE than it was in the control injections. The general reaction of the animal to adrenine was greatly changed during the experiment. In the controls its only obvious effect was acceleration of the heart and a slight pallor of the mucous membrane of the mouth and



Figs. 1–8. Each figure shows the action of APE on the adrenine response of the denervated heart before and after thyroidectomy, compared with the action of thyroxine or desiccated thyroid. Figures 5 and 7 show, in addition, the effect of removal of the anterior lobe of the pituitary. For the conditions in each figure consult the key to the graphs given in the central space.

the skin of the nose, which in a minute had passed away. After APE the same dose of adrenine produced much more definite changes. There was an extreme pallor of the mucous membrane and skin which lasted over a minute and then gradually changed to a bluish hue. The second minute after injection the animal was near collapse, with marked dyspnea. This was accompanied by retching movements. The time taken for the effect to wear off was several minutes. In the other three cats smaller increases in sensitivity occurred and the marked changes in the general body response were lacking.

These experiments, to test the effect of APE on adrenine sensitivity, could not be continued for a longer period than 14 days, as the supply of extract was limited. Within that period we could not determine whether the maximal increase had been reached; nor could we learn whether further administration would be followed by a remission of the response, as had

TABLE 2

Change in weight, basal metabolic rate (B. M. R.), basal heart rate and adrenine response after APE

CAT NUMBER	TIME APE ADMINISTERED	CHANGE IN WEIGHT AFTER APE	INCREASE IN B. M. R.	INCREASE IN BASAL HEART RATE	INCREASE IN HEART RESPONSE TO ADRENINE OVER CONTROL RESPONSE—DURING MINUTE AFTER INJECTION
	days	*kgm.*	*per cent*	*beats per minute*	
4	7	0.50+		24	6
13	7	0.20+		20	7
10	14	1.25+	22	24	9
11	9	0.10+	20	16	18
25	13	0.20−	3	0	0

been observed by Friedgood (1934) in studies of the metabolic rate of guinea pigs receiving APE.

A definite increase of the basal heart rate also was observed after APE in the four animals which showed an increased adrenine response (table 2). In the fifth (cat 25), which had been unresponsive to the extract, the basal heart rate was unchanged. As with the adrenine response, the effect of APE on the basal rate was not immediate but took several days to appear. The maximal increase usually was observed at the time of maximal adrenine response.

Determinations of the basal metabolic rate were made on three of the above animals (cats 10, 11 and 25) before and after administration of APE. Increases of 20 and 22 per cent were observed in two (cats 11 and 10) but in the third, which had shown no increase of basal heart rate or adrenine response, the basal metabolic rate was unchanged (table 2). A small percentage of guinea pigs are likewise refractory from the begin-

ning to the thyrotropic effects of this anterior pituitary extract (Friedgood, 1934).

Some increase of weight was observed during the administration of APE in four animals. In this respect cat 25 was again exceptional in that it showed no increase (table 2).

The effect of thyroidectomy. It is possible that the stimulating action of APE on the basal rate of the denervated heart and on its sensitivity to adrenine is an indirect effect produced by changes in the activity of other glands. Evidence has been presented that both the basal rate and sensitivity can be affected by changes in the thyroid secretion (Sawyer and Brown, 1935). Furthermore, the APE used in the present research was known to have a thyrotropic component (Friedgood, 1934). Experiments were therefore made after removal of the thyroid to learn if the increased basal rate and adrenine sensitivity after APE were due to overactivity of this gland. In some of these experiments APE was given to the animals before and after thyroidectomy, and in others only after thyroidectomy.

From three of the above animals (figs. 1, 2 and 3), which had shown an increased adrenine sensitivity and basal rate after APE, the thyroid gland was removed and the response to the extract again tested. After removal of the gland all three animals showed a decrease in the sensitivity to adrenine, as had been previously observed by Sawyer and Brown. When the adrenine response was well below the control value, administration of APE was begun again. It was given in the same dose and continued for the same length of time as before thyroidectomy. In none of these animals was there an increase of the adrenine response as had previously been noted when the thyroid was intact. In two cats (4 and 11) the response decreased still further after the administration of APE, while in the third (13) it remained approximately the same as when the extract had been first injected.

The objection might be raised to the above type of experiment in which APE was administered for two periods, that the animals became refractory after the first administration and would not respond to the second series of injections. To eliminate this possibility experiments were performed on three animals in which APE was given only after thyroidectomy. In these cats (5, 14 and 30) the thyroid was removed as soon as the control adrenine response had been established. APE was then administered for 10, 8 and 8 days respectively. At the end of this time in all three animals the adrenine response was less than the original control, but it was slightly higher than the lowest value observed after thyroidectomy, before the APE injections were begun (figs. 6, 7 and 8).

It is difficult to draw any definite conclusions regarding the effect of APE on the basal heart rate after thyroidectomy, since in this state it

varies often from day to day. In most cases, however, there is a slight increase after APE has been given for several days, although the change is less than that observed in animals with thyroids intact. For example, in cat 13 before thyroidectomy, administration of APE for 8 days raised the basal rate 20 beats per minute, whereas after thyroidectomy a similar dose of extract was followed by an increase of only 8 beats.

The effect of thyroxine and thyroid feeding. Since the action of APE on the basal heart rate and sensitivity to adrenine appears to be dependent on the presence of the thyroid, it was considered advisable to compare the relative effectiveness of APE and thyroid administration as a means of increasing the adrenine response. Previously Sawyer and Brown found that the administration of thyroxine was followed by a slight increase in the sensitivity to injected adrenine. They used a semi-purified thyroxine which was very insoluble and was administered by mouth. It was tried in only two animals.

To six of the cats mentioned above (4, 13, 10, 11, 5 and 14), in which the effect of APE on adrenine sensitivity had been observed, thyroxine or a thyroid preparation was administered. In two of these animals (cats 4 and 13) thyroxine was given orally in doses of 8 to 15 mgm. daily for 5 and 14 days respectively. In animal 4 thyroxine produced a rise in the adrenine response of about the same order of magnitude as that produced by the APE (fig. 1), while in animal 13 no increase was obtained (fig. 2). In the other four cats desiccated thyroid, which is a much more soluble product, was used. It was given in doses of 128 to 264 mgm. for 8 to 14 days. The desiccated thyroid in large doses proved to be a much more effective means of augmenting adrenine sensitivity than was thyroxine. In all four animals its administration was followed by a definite increase in the response to adrenine. This increase is shown in table 3; it is expressed as the difference in the number of beats, in the minute immediately after the adrenine injections, between the control experiments and those with thyroid. Cat 11 is of special interest. As previously mentioned, this animal showed an unusually marked reaction to adrenine after APE. A similar response was noted after feeding desiccated thyroid for 14 days. At this time the injection of the standard adrenine again produced an extreme pallor of the mucous membranes which lasted several minutes. This was accompanied by dyspnea and vomiting movements. Cat 11 was the only animal which showed such a severe reaction to adrenine after APE and thyroxine.

Marked increases in basal heart rate took place in all the animals after administration of the desiccated thyroid. This rise is greater than that seen after APE (compare tables 2 and 3).

The effect of ablation of the anterior lobe of the pituitary. An attempt was made to test the effect of ablation of the anterior lobe of the pituitary on

adrenine sensitivity. A number of animals were operated upon but only two survived for more than a few days.

The method of ablation was as follows. A midline incision was made in the upper part of the neck and a dissection was done back to the hard palate and carried forward to the basi-sphenoid bone. Care was taken to separate the mucous membrane of the nasopharynx from the bone without puncturing it. At a point midway between the two auditory bullae and just anterior to them a small hole was drilled through the bone by means of a dental drill. The field was kept free of blood by suction. When the pituitary gland was revealed the anterior lobe was removed by suction. Probably a small portion of the posterior lobe was also removed. The most difficult part of the procedure was to keep the nasopharyngeal mucous membrane intact and thereby maintain asepsis.

TABLE 3

Changes in basal heart rate and adrenine response after thyroxine and desiccated thyroid

CAT NUMBER	TIME THYROID ADMINISTERED	INCREASE IN BASAL HEART RATE AFTER THYROID	INCREASE IN HEART RESPONSE TO ADRENINE OVER CONTROL RESPONSE—DURING MINUTE AFTER INJECTION
	days	*beats per minute*	
4*	5	0	10
13*	5	0	0
10	9	36	9
11	14	32	9
5	8	32	30
14	10	24	14

* Animals given thyroxine (8 to 12 mgm.) daily; others given 128 to 264 mgm. desiccated thyroid daily.

Cat 25, the only one which did not respond to APE, survived the removal of the anterior pituitary. In this animal, a week after the operation, there was a lessening of the adrenine response. At the end of two weeks there was a still further decrease; it was then less than half of what it had been after the control injections (fig. 5). The diminished response persisted for another two weeks, when the animal was given desiccated thyroid for 4 days. This restored the adrenine reaction (dash line and circles, fig. 5) to practically that of the control experiments. A test made 10 days later showed that response had again fallen to the low level seen previous to the thyroid feeding.

The second cat which survived removal of the anterior pituitary was number 14, from which the thyroid was removed at the beginning of the experiment. In this animal there was the typical depression of sensitivity

after thyroidectomy. Subsequent removal of the anterior pituitary caused very little change in the response.

CONCLUSIONS. Previous evidence has shown that changes in the activity of the thyroid results in changes in sensitivity to a standard dose of adrenine. From the above experiments it is obvious that APE also influences adrenine sensitivity. Administration of this extract causes an increase in the heart response to adrenine, whereas removal of the anterior pituitary diminishes it. If the thyroid gland is removed, however, the stimulating action of the APE is abolished. It seems likely, therefore, that the anterior pituitary does not directly affect the sensitivity of the denervated heart to adrenine but acts on it by producing an increased activity of the thyroid. This supposition is supported by the fact that there is a close similarity between the action of APE and thyroxine and the fact that the extract causes an increase in the basal metabolic rate.

SUMMARY

A study was made of the effect of anterior pituitary extract on the response of the denervated heart to adrenine.

It was found that the denervated heart undergoes no noteworthy change of sensitivity to adrenine after the first days following section of its nerve supply (table 1).

In four of five cats the administration of anterior pituitary extract (APE) for 1 to 2 weeks was accompanied by an increase in the sensitivity of the denervated heart to a standard dose of adrenine (figs. 1, 2, 3, 4, and table 2). This increase did not appear for 5 to 8 days after the extract began to be administered. In two of four cats whose metabolism was determined, there were 20 and 22 per cent rises in the metabolic rate after APE (table 2).

A marked increase in basal heart rate was also observed in the same four animals after APE (figs. 1, 2, 3, 4, and table 2).

In three of the above animals the thyroid was removed. After this operation a second administration of APE caused no increase in the adrenine response (figs. 1, 2 and 3). Similarly, in three other animals in which thyroidectomy was performed at the beginning of the experiment there was no increase in sensitivity to adrenine after APE (figs. 6, 7 and 8).

APE caused a slight increase in the basal heart rate of the thyroidectomized animals, but its effect was much less than when the thyroid was intact.

After the administration of thyroid there was a marked increase in the adrenine response of the denervated heart in five animals (figs. 1, 3, 4, 6, 7, and table 3). In these animals thyroid feeding also caused a considerable increase in the basal heart rate (table 3).

Removal of the anterior lobe of the pituitary in one animal with the thyroid intact was followed by a definite decrease in the sensitivity of the denervated heart to adrenine (fig. 5).

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